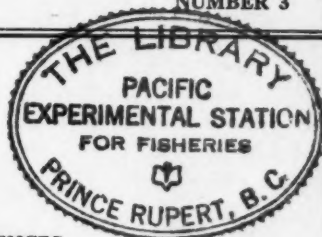


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TRANSPORT OF MATERIAL IN SOURCES FOR SPECTROSCOPIC ANALYSIS¹

By G. O. LANGSTROTH² AND D. R. McRAE²

Abstract

A study of the transport of material in the condensed d-c. spark discharge has been made, with the object of placing quantitative spectroscopic methods of analysis on a less empirical basis and thus permitting, (a) the recognition of certain conditions of source operation which lead to unreliable determinations, and (b) the determination of some of the criteria to be fulfilled for maximum precision in analytic measurements.

Variations in the transport phenomena, as indicated by variations in the relative distributions of emitting atoms of various elements along the discharge axis on the addition of certain substances to a sample, appear to be closely connected with the ionization potentials and masses of the elements, and with the ionization potentials of other particles present in the discharge. The data indicate the necessity for the use of a spectroscopic buffer, and provide certain criteria for the choice of the buffer and of the internal standard element.

The exact nature of the mechanism of transport is uncertain, but various considerations indicate that neither the motion of ions under the influence of the field nor diffusion in the ordinary sense is sufficient to account for observed features.

The increasing use of quantitative spectroscopic methods of analysis in various fields of research and in industry makes it highly desirable to obtain some understanding of the basic phenomena on which the methods depend. Studies of the mechanisms of sources serve to place the methods on a well understood, rather than on a purely empirical, basis. As a result, those conditions of source operation which lead to unreliable results may be recognized and avoided, and considerable information is made available concerning the appropriate method of attack on special analytic problems.

Previous investigations (4) have indicated that the excitation in condensed spark discharges produced by circuits having a fairly long oscillation period (10^{-4} sec.) may be considered as thermal in character, just as in the freely burning arc. The present article is concerned with a study of the second of the important source mechanisms, *viz.*, the transport of material through the discharge gap after it has been released from the electrode. The investigations have been limited to a study of the d-c. spark discharge, but there can be little doubt that the inferences drawn from it apply in some degree to any of the ordinary forms of spark discharge. In the authors' experience, condensed spark sources have been found to be capable of more reliable deter-

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minations than the less commonly used arc sources; hence they merit more detailed study.

There is some evidence that the transport phenomena in arc sources involve an equilibrium between ordinary thermal diffusion of material from the region of the electrode and movement of ions of the material under the influence of the electric field (5). As is indicated in Section 3 of the present article, transport of material in spark sources involves more complex phenomena.

For the most part the investigations consisted of a study of the relative distribution of intensity along the lengths of spectral lines of various elements. Under the conditions employed, the intensity distribution for an element corresponded closely to the time integrated distribution of the emitting atoms of the element along the axis of the discharge.

In general, the intensity ratio of two lines due to different elements was found to be different at different points along their length. The relation between the ratio at a point near the loaded electrode, and that, say, at the centre of the discharge gap, was not constant, but could be altered by the addition of certain substances to the material placed on the electrode. The extent to which it was altered appeared to depend on the ionization potentials and masses of the two elements, and on the ionization potentials associated with the added substance. The observed variations are undoubtedly due to variations in the transport phenomena.

From the viewpoint of practical analysis, it is important to recognize that such variations may occur. *Unless appropriate precautions are taken*, the intensity ratios—and hence the determined concentrations—may vary with variations in the extraneous composition of the samples, even though the amounts of the internal standard and the investigated element present remain constant. Such variations lead to unreliable results. It should be emphasized that this statement is made purely on the basis of observed variations in the transport phenomena, and does not take into consideration variations which may occur in the mechanism responsible for the release of material from the electrode. The latter factor may be expected to introduce further complications.

On the basis of the present results, the precautions which should be taken to minimize the effects of variations in transport phenomena are; (a) the use of an appropriate "spectroscopic buffer", and (b) the choice of an appropriate internal standard element.

Section 1 deals with a description of the experimental procedure; the experimental results are given in Section 2. Section 3 is concerned with some considerations of the mechanism of spark discharge from the viewpoint of spectroscopic analysis. Section 4 deals mainly with the practical problem of the effects produced by variations in the transport phenomena, and with the means of minimizing them.

1. Experimental Procedure

The spark discharge was produced by the d-c. circuit (4) used for making analyses. A switch inserted in the circuit permitted single sparks to be

taken at any desired intervals. The calculated oscillation period of the circuit ($C = 0.12 \mu F$, $L = 2000 \mu H$, $R \sim 3 \Omega$) was about 10^{-4} sec.; this value may be expected to approach the actual value closely (7, p. 83; 9, p. 342).

For the most part the electrodes consisted of a copper point-plane pair (separation, 2.5 mm.) as previously described (4). An image of the discharge (magnification, $\sim 5\times$) was focused on the slit of a quartz Hilger E2 spectrograph, the axis of the discharge being parallel to the slit. Since this was done with a quartz-fluorite achromatic lens, the distribution of intensity along the length of a spectral line corresponded closely to the time integrated axial distribution in the discharge of atoms emitting the particular wavelength of light.

A portion (1/50 cc.) of a special solution (see Table I) was dried on the plane electrode (9×10 mm.). The electrodes were placed in position, and an exposure consisting of six single sparks, each of which was made to strike a fresh surface on the plane, was taken. The procedure was repeated with the addition of various chosen substances to the standard solution. The intensities at three or four points along the lengths of chosen lines of the various elements were determined for each exposure, and the intensity ratios at the various axial points calculated. Intensity measurements were made by the standard method employing a Moll microphotometer, calibration marks being put on each plate with the aid of a step-slit and quartz band lamp.

There was no evidence of appreciable self-reversal or self-absorption in the lines chosen for the investigation. As is indicated in Table III, all the examined transitions, except those for boron, aluminium, and possibly manganese, are associated with final levels which lie at some distance above the ground level. Moreover the amounts of the elements present in the discharge were small. The amount of tin present on the electrode, for example, was only 2×10^{-7} gm. per sq. mm., and the amount removed by a single spark was less than 8×10^{-8} gm.

The standard solutions, to which the additions were made, contained two or more of the elements given in Table I. The water of crystallization has been omitted from the chemical formulas. V , M , and b.p. denote respectively the ionization potential, atomic mass, and boiling point. The last named

TABLE I
ELEMENTS FOR STANDARD SOLUTIONS

Element	Conc., gm. per cc. $\times 10^3$	V , volts	M	B.p., °C.	Compound	Characteristics
Hg	2	10.34	200.6	357	HgCl ₂	B.p. 301° C.
Zn	1	9.36	65.4	907	ZnCl ₂	B.p. 732° C.
Cd	2	8.96	112.4	—	CdCl ₂	B.p. 960° C.
B	0.5	8.29	10.8	2250	H ₃ BO ₃	Decomp. 185° C.
Mg	1	7.61	24.3	1110	Mg(NO ₃) ₂	—
Mn	0.8	7.41	54.9	1900	MnCl ₂	B.p. 1190° C.
Pb	1	7.38	207.2	1613	Pb(NO ₃) ₂	Decomp. 357° C.
Sn	1	7.30	118.7	2270	SnCl ₂	Decomp.
Al	1	5.96	27.0	1800	Al ₂ (SO ₄) ₃	Decomp. < 770° C.

value is given in order to suggest the range covered in the investigations, and to indicate that stability and ease of evaporation play little part in the phenomena to be discussed later. Some of the substances change their form in solution and so are not present on the electrode in the stated compound; moreover, many of them, at least, may be changed during the passage of a single spark.

TABLE II
SUBSTANCES FOR ADDITION TO STANDARD SOLUTIONS

Substance	Conc., gm. per cc. $\times 10^2$	Lowest V, volts	Characteristics
Sugar	4	11.22 (C)	Decomp. easily
NH ₄ Cl	0.5-5	12.96 (Cl)	Decomp. 350° C.
H ₃ BO ₃	4	8.29 (B)	Decomp. 185° C.
CaCl ₂	4	6.09 (Ca)	B.p. 1600° C.
Li tartrate	4	5.37 (Li)	Decomp. easily
KNO ₃	4	4.32 (K)	Decomp. 400° C.
NaK tartrate	4	4.32 (K)	Decomp. easily
KCl	0.5-5	4.32 (K)	Sublimes 1500° C.

While the intensity distributions were usually determined for the lines given in Table III, other lines were sometimes examined as well. They gave essentially the same results. Table III gives the initial and final levels (with their energies in cm.⁻¹) of the commonly examined transitions, as well as the energies of the ground levels of the atoms (1, 3).

TABLE III
LINES COMMONLY INVESTIGATED

Element	λ , Å	Initial state	Final state	Ground state
Hg	3131	$\left\{ \begin{array}{l} {}^3D_1 \text{ (6s3d)} \\ {}^1D_2 \text{ (6s6d)} \end{array} \right\}$	$\left\{ \begin{array}{l} {}^3P_0^o \text{ (6s6p)} \\ {}^3P_1^o \text{ (6s6p)} \end{array} \right\}$	84179
Hg ⁺	2847	${}^3S_{1/2} \text{ (5d}^2\text{6s)}$	${}^3P_{3/2}^o \text{ (5d}^2\text{6p)}$	151280
Zn	3302	${}^3D_2 \text{ (4d)}$	${}^3P_1^o \text{ (4s4p)}$	75769
Cd	3403	${}^3D_1 \text{ (5s5d)}$	${}^3P_0^o \text{ (5s5p)}$	72539
Cd ⁺	2748	${}^3S_{1/2} \text{ (4d}^2\text{6s)}$	${}^3P_{3/2}^o \text{ (5p)}$	136377
B	2497	${}^3S_{1/2} \text{ (3s)}$	${}^3P_{3/2}^o \text{ (2s}^2\text{2p)}$	0
Mg	2780	${}^3P_{1,2} \text{ (3p}^2)$	${}^3P_{2,1}^o \text{ (3s3p)}$	61672
Mg ⁺	2791	${}^3D_{3/2} \text{ (3d)}$	${}^3P_{3/2}^o \text{ (3p)}$	121267
Mn	4034	—	—	—
Pb	3639	$2^3_1[6p({}^3P_{1/2})7s]$	${}^3P_1 \text{ (6p}^2)$	59821
Sn	3175	${}^3P_1^o[5p({}^3P_{1/2})6s]$	${}^3P_2 \text{ (5p}^2)$	59192
Al	3082	${}^3D_{3/2} \text{ (3d)}$	${}^3P_{3/2}^o \text{ (3s}^2\text{3p)}$	48281

2. Results

It became apparent early in the investigation that any description of the changes in the relative distribution of intensity for two elements, brought about by addition of substances to a standard sample, must involve a con-

sideration of the masses and the ionization potentials of the elements. In order to separate effects due to mass difference from effects due to ionization potential difference, experiments were carried out with (a) a series of elements having different masses but comparable ionization potentials, and (b) a series having different ionization potentials but comparable masses. Since the occurrence of such series is not common in the periodic table, these data must be somewhat limited. Results of typical experiments are given in Tables IV and V.

The tabulated values are the intensity ratios for various pairs of elements at different distances, D , from the loaded plane electrode. For the purposes of comparison of the relative distributions of intensity, the ratios at the point nearest the plane have been set equal to 1.00. This is permissible since the relative distributions are not appreciably affected by such changes in the concentrations as would be required to give an observed value of 1.00 at

TABLE IV
CHANGES IN THE RELATIVE DISTRIBUTION OF INTENSITY FOR ELEMENTS HAVING COMPARABLE IONIZATION POTENTIALS BUT DIFFERENT MASSES

M, ratio = V, ratio =	Sn-Pb			Mn-Pb			Mg-Pb		
	119/207 7.30/7.38			54.9/207 7.41/7.38			24.3/207 7.61/7.38		
	D , mm.			D , mm.			D , mm.		
Addition	0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9
None	1.00	0.96	1.20	1.00	1.20	1.36	1.00	0.98	1.31
Sugar	1.00	0.96	1.02	1.00	1.39	1.52	1.00	1.21	1.34
H ₃ BO ₃	1.00	1.00	1.24	1.00	1.27	1.46	1.00	1.26	1.46
Li tart.	—	—	—	1.00	1.27	1.37	1.00	0.93	0.81
KCl	1.00	1.12	1.10	1.00	1.10	1.15	1.00	0.69	0.74

TABLE V
CHANGES IN THE RELATIVE DISTRIBUTION OF INTENSITY FOR ELEMENTS HAVING COMPARABLE MASSES BUT DIFFERENT IONIZATION POTENTIALS

$\frac{M}{V}$, ratio * = $\frac{V}{V}$, ratio =	Hg-Pb			Cd-Sn			Mg-Al			
	201/207 10.3/7.38			112/119 8.96/7.30			24.3/27.0 7.61/5.96			
	D , mm.			D , mm.			D , mm.			
Addition	0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9	
None	1.00	0.78	0.94	1.00	0.91	0.90	1.00	0.98	0.93	
Sugar	1.00	0.85	0.91	1.00	1.07	1.07	1.00	0.85	0.85	
H ₃ BO ₃	1.00	0.84	0.87	1.00	0.95	0.92	1.00	0.96	0.99	
Li tart.	1.00	0.51	0.38	1.00	0.79	0.69	1.00	0.60	0.54	
KCl	1.00*	0.88	0.39	0.38	1.00	0.49	0.49	1.00	0.54	0.58

* This ratio had a maximum slightly closer than 0.4 mm. to the plane electrode when potassium chloride was added to the solution.

this point. It is equivalent to changing the concentrations of some elements by small factors. In view of this procedure, the results reflect only the variations in the transport phenomena, and are not directly concerned with variations in the mechanism responsible for the release of material from the electrode.

Table VI contains data for a series of elements chosen for their ionization potential differences without respect to their mass differences. The relative distribution for any two of the elements may of course be obtained from the given data. The mode of representation is the same as that for Tables IV and V.

The results of all the experiments may be generalized as follows:

(a) Lines of different elements have, in general, different intensity distributions along their length.

(b) The relative distributions depend on the ionization potentials and masses of the elements, and on the ionization potentials of other atoms present in the discharge.

(c) When easily ionized atoms (e.g., potassium) are present in the discharge, the distribution of an element relative to that of a more easily ionized element of comparable mass falls off more sharply toward the unloaded electrode than it does when easily ionized atoms are absent (cf. Table V). A similar statement may be made concerning the distribution of a light element relative to that of a heavy element of comparable ionization potential. The effect of mass difference is, however, marked only for very light elements (e.g., magnesium). It has only a small influence in the case of manganese with a mass of 54.9 (cf. Table IV).

The following remarks may be made concerning particular points brought out by the data.

(1) Since the discharge contains a considerable number of copper atoms liberated from the electrodes, *marked* changes in the relative distributions of intensity are to be expected only on addition of atoms having an ionization potential lower than that of copper (7.68 v.) i.e., the presence of the copper atoms tends to "stabilize" the discharge. This point is illustrated by the curves of Fig. 1 and the data of Tables IV, V, and VI.

(2) If the number of copper atoms released from the electrodes is decreased, as it might reasonably be by the presence of an appreciable layer of salts over the electrode, the relative distributions should be changed somewhat owing to the partial substitution of some other atoms for copper in the discharge. It may be seen from Table VI, for example, that addition of a substance associated with high ionization potentials to a standard solution tends to change the relative distributions in the direction expected for a partial substitution of difficultly ionized atoms for copper.

(3) The relative distribution of two elements depends on the composition of the standard solution, since other elements present may be considered as

TABLE VI
CHANGES IN THE RELATIVE DISTRIBUTION OF INTENSITY FOR VARIOUS ELEMENTS ON ADDITION OF DIFFERENT SUBSTANCES

Elements in std. solution	Addition	Hg-Sn			Zn-Sn			Cd-Sn			B-Sn		
		D, mm.			D, mm.			D, mm.			D, mm.		
		0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9
Hg, Cd, Sn Mg, Pb, Al Mn	None	1.00	0.80	0.78	—	—	—	1.00	0.84	0.84	—	—	—
	Sugar	1.00	0.88	0.88	—	—	—	1.00	1.07	1.07	—	—	—
	H ₂ BO ₃	1.00	0.92	0.78	—	—	—	1.00	0.95	0.92	—	—	—
	KCl	1.00*	0.90	0.35	—	—	—	1.00	0.49	0.49	—	—	—
Hg, Zn, Sn B	None	1.00	0.87	0.97	1.00	1.11	1.04	—	—	—	1.00	1.12	1.48
	KCl	1.00*	0.88	0.41	1.00	0.61	0.56	—	—	—	1.00	0.63	0.65
Elements in std. solution	Addition	Mg-Sn			Mn-Sn			Pb-Sn			Al-Sn		
		D, mm.			D, mm.			D, mm.			D, mm.		
		0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9	0.4	1.2	1.9
Hg, Cd, Sn Mg, Pb, Al Mn	None	1.00	1.00	1.02	1.00	1.24	1.14	1.00	1.03	0.83	1.00	1.04	1.12
	Sugar	1.00	1.27	1.32	1.00	1.44	1.47	1.00	1.04	0.96	1.00	1.48	1.55
	H ₂ BO ₃	1.00	1.27	1.18	1.00	1.27	1.19	1.00	0.99	0.81	1.00	1.31	1.20
	KCl	1.00	0.63	0.70	1.00	0.99	1.06	1.00	0.91	0.93	1.00	1.16	1.18
Hg, Zn, Sn B	None	—	—	—	—	—	—	—	—	—	—	—	—
	KCl	—	—	—	—	—	—	—	—	—	—	—	—

* These ratios had a maximum slightly closer than 0.4 mm. to the plane electrode on the addition of potassium chloride to the solution.

additions. The observed differences between the distributions for simple and for complex standard solutions are always in the expected direction.

(4) The data of Table VI, unlike those of Tables IV and V, include in many cases the resultant effect of mass difference and ionization potential difference. The behavior of the mercury-tin, cadmium-tin, and lead-tin ratios is almost entirely due to the latter factor, since all are fairly heavy elements. It is to be noted that the distribution lead-tin is little affected by additions, as expected from the fact that lead and tin have comparable ionization potentials. For the zinc-tin and manganese-tin ratios the mass difference effect begins to be felt (cf. Table IV, manganese-lead), but is still relatively small. For the magnesium-tin ratios, the behavior is due almost entirely to the mass difference effect, since magnesium and tin have comparable ionization potentials. For the aluminium-tin ratios both effects are present, but operate in opposite directions and so tend to compensate each other. As a result, the distribution does not change markedly on additions, although the mass difference effect appears to be somewhat the stronger with potassium chloride additions (cf. Fig. 1, aluminium-lead). For the boron-tin ratios both effects are present and operate in the same direction. The changes in the relative distribution

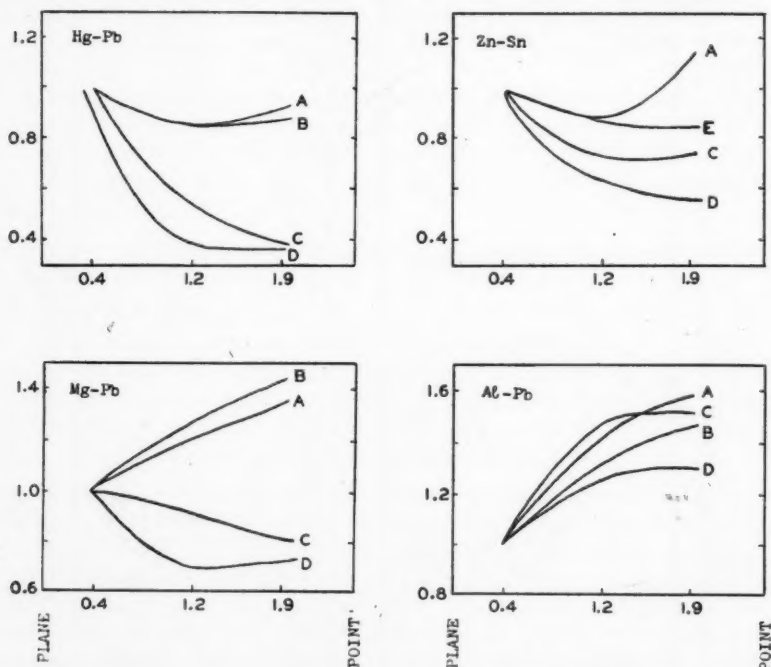


FIG. 1. The relative distributions of intensity for four typically different pairs of elements on addition of various substances to standard samples. The ordinates represent intensity ratios; the abscissas, distances from the loaded electrode in millimetres. Additions: A = sugar, B = H_3BO_3 , C = Li. tart., D = KCl, E = $CaCl_2$.

are comparable to those for the mercury-tin ratios although the ionization potential difference is much less.

The changes which occur in the relative distributions on the addition of substances with successively lower ionization potentials are shown in Fig. 1 for four typical pairs of elements. These are, (a) mercury-lead, ionization potential difference effect alone, (b) magnesium-lead, mass difference effect alone, (c) zinc-tin, both effects with the ionization potential effect predominating, (d) aluminium-lead, both effects operating in opposite directions.

The results of certain other experiments deserve some mention since they support the interpretations already given.

(a) Experiments in which graphite rather than copper electrodes were used, but otherwise similar to those described, gave results consistent with the data of this section.

(b) Experiments in which potassium nitrate or sodium potassium tartrate, instead of potassium chloride, was added to the standard solutions, showed that the effect of the potassium salt addition was not connected with the stability or ease of evaporation of the salt on the electrode.

(c) Experiments in which the amount of the added salt (ammonium chloride or potassium chloride) was varied, showed that the observed relative distributions are not dependent on the amount of the salt added within the limits of the investigation. The limiting values of the added concentrations were 0.5×10^{-2} and 5×10^{-2} gm. per cc. of potassium chloride or ammonium chloride.

In several experiments the temperatures at different points along the discharge axis were determined from the intensity ratio of 3262/3175, tin, in the way previously described (4). The results, given in Table VII, indicate that the temperature of neutral atoms is essentially constant along the axis, although there is a suggestion that with ammonium chloride addition the temperature is slightly higher near the loaded electrode.

TABLE VII
TEMPERATURE MEASUREMENTS ALONG THE DISCHARGE AXIS.

Addition	Observations	Average intensity ratio 3262/3175, tin					
		0.4 mm.	T, °K.	1.2 mm.	T, °K.	1.9 mm.	T, °K.
None	1	1.06	9400	1.09	9800	1.02	8900
NH ₄ Cl	7	1.11	10100	1.07	9500	1.07	9500
KCl	3	1.04	9200	1.05	9300	1.04	9200

On the other hand, the intensity ratio of 2791 Mg⁺/2780 Mg decreased more or less regularly toward the unloaded electrode under all conditions. The observed values for various distances from the plane electrode are given in Table VIII. It is clear from these figures that the ionization process is not

in an equilibrium state throughout the spark gap. A possible explanation is indicated in the following section.

TABLE VIII
CHANGES IN THE RELATIVE DISTRIBUTIONS OF SPECTRAL
LINES OF THE IONIZED AND NEUTRAL ATOM (Mg)

Addition	Distance from plane electrode, mm.		
	0.4	1.2	1.9
None	4.5	2.9	3.0
Li. tart.	1.9	0.7	0.8
KCl	2.0	0.9	0.8

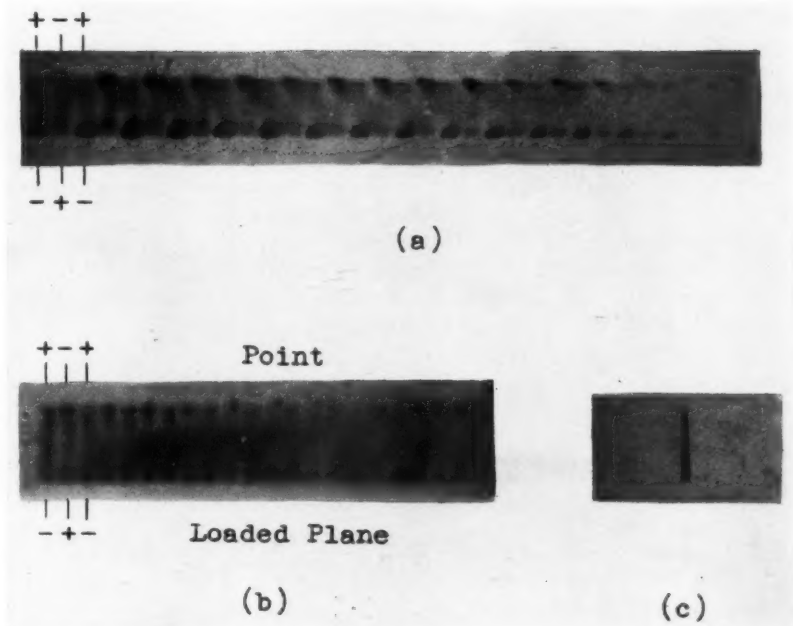
The Hg⁺ line $\lambda 2847$, and the Cd⁺ line $\lambda 2748$, were quite strong in the neighborhood of the loaded electrode, but decreased so rapidly in intensity that measurements could not be made near the centre of the gap. The decrease in intensity is more marked relative to the atomic lines, than is the case with magnesium.

3. The Mechanism of Spark Discharge from the Viewpoint of Spectroscopic Analysis

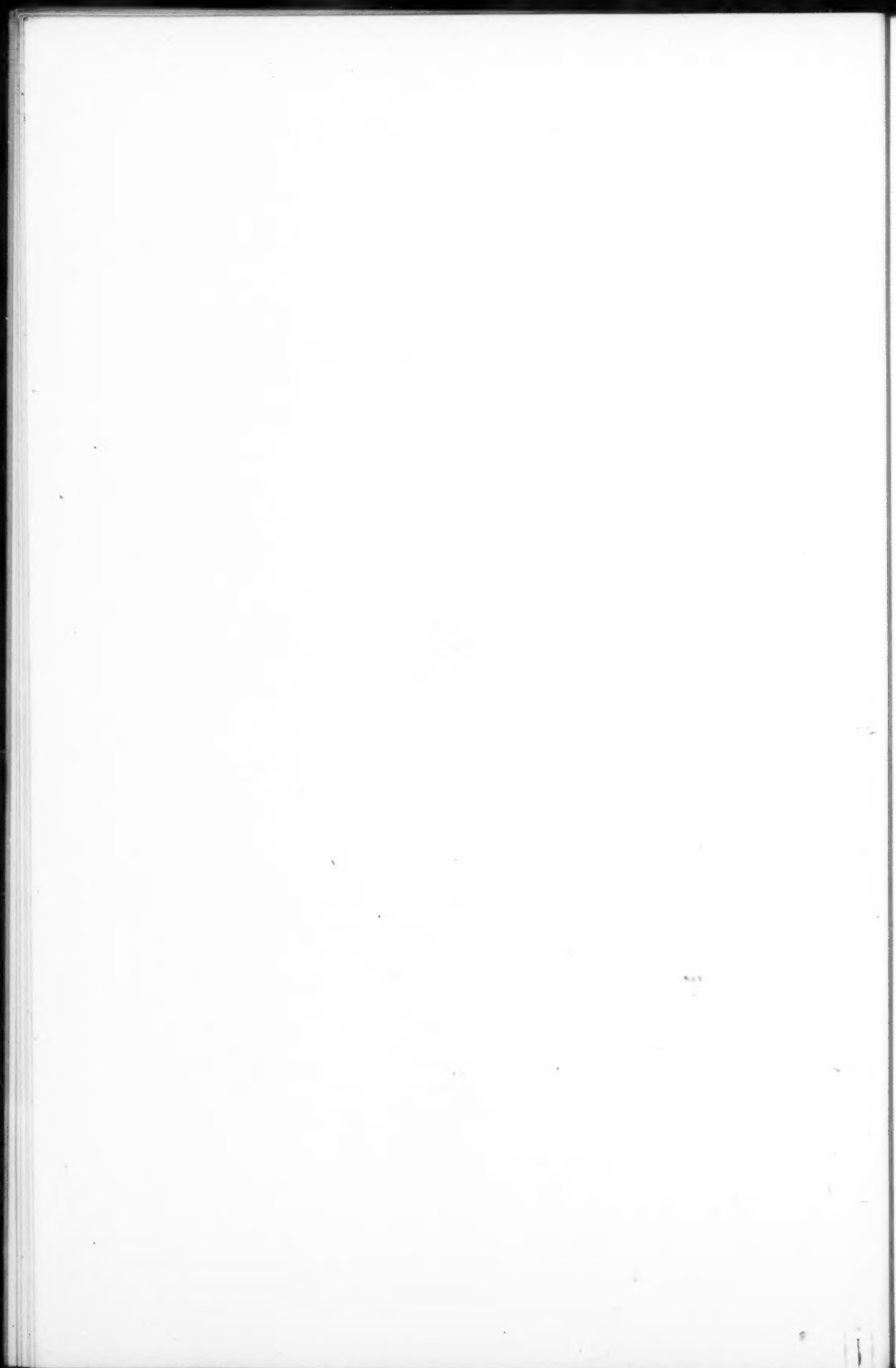
If the persistence of temperature excitation is neglected, each single spark produced by the commonly used circuit containing the gap, a capacity, and an inductance may be considered to consist of several separate discharges, each of which is associated with a half oscillation of the circuit. There may be from 20 to 30 such discharges per spark (see Plate I, and also the excellent reproductions in Reference 7). The oscillation period of the circuit is little affected by changes in the nature of the spark gap (7, 9), and may be quite closely calculated from a knowledge of the circuit constants (7). If the inductance in the circuit described in Section 1 be reduced to that of the leads, the oscillation period decreases from about 10^{-4} to 10^{-6} sec.

The potential difference across the spark gap during these discharges is not high (8). On each half oscillation except the first, it has an initial value of about 300 volts, but falls rapidly and reaches a value of about 35 volts in a small fraction of a half period; it remains at this value for the duration of the half oscillation. Neither the time relations nor the magnitudes of these voltages are markedly affected by changes in the gap length or in the oscillation period. In view of the magnitudes of the voltages, it is probable that each of the discharges which constitute a single spark begins as a glow discharge, but rapidly changes to an arc-like form as the cathode becomes locally heated by positive ion impact.

The spectroscopic analyst is primarily interested in the spread of the vapor of the electrode material through the gap, and with its excitation and the subsequent emission of light. When a spark is viewed with the aid of a rotating mirror, characteristic luminous streamers may be seen to start off from both electrodes at each half oscillation, and to proceed with diminishing velocity toward the centre of the gap (7, 9, 10, 11). The streamers are associated in some way with the vapor of the electrode material, since they radiate its spectrum exclusively.



Rotating mirror photographs of single sparks produced by a circuit having a period of approximately 10^{-4} sec., showing the separate discharges which take place at each half oscillation. Owing to the small rotational speed of the mirror, the streamers are not so strongly curved as they appear with higher speeds, i.e., when shorter period sparks are examined. (a) Photograph of a single spark between copper point electrodes. (b) Photograph of a single spark between a copper point and a copper plane electrode loaded with sodium potassium tartrate as for analysis. Note the progress of a characteristic luminosity arising from the plane electrode; this luminosity is probably to be associated with emission of light from the atoms of the load material. (c) Photograph taken with the mirror stationary, in order to indicate the width of the image of the slit which was placed immediately before the spark gap in (a) and (b). The speed of rotation of the mirror was not the same in (a) and (b); in both, the discharges occurred at intervals of 5×10^{-5} sec.



It is difficult to see how the streamers could result from the motion of electrons through a more or less stationary cloud of vapor. The passage of electrons from the cathode takes place during the greater part of the half oscillation, and should produce a streamer having a time width in the rotating mirror photograph, of approximately half a period at the cathode. The streamers may be obtained, however, as quite sharp curved lines of a time width of only a small fraction of a half period. Furthermore, the streamers start from *both* electrodes, although those from the cathode are usually the more prominent. A consideration of the velocity of the streamers leads to a similar conclusion. The velocity with which the envelope of the streamers advances depends on the metal used for the electrodes and on the constants of the circuit, but may be as low as 20,000 cm. per sec. If the field in the arc-like phase of the discharge is 20 v. per cm. (cf. fields in metal arcs (12, p. 587 ff)), and if the mobility of an electron be taken as 24,000 cm. per sec. per v. per cm. (12, p. 526), the electron drift velocity is 500,000 cm. per sec.; *i.e.*, it is higher than the minimum velocity of the streamers, and in view of what has been said concerning the constancy of the gap voltages, should not change very much when the conditions are changed.

The motion of positive ions under the influence of the field can play little part in transport of material through the gap, or in the development of the streamers. The ions, with a mobility of about 1.4 cm. per sec. per v. per cm. (14), have a drift velocity of about 28 cm. per sec. in a field of 20 v. per cm. Hence, in 5×10^{-6} sec., the distance traveled under the influence of the field is only about 0.001 mm. Well developed streamers have been observed, however, with circuits having a half period of only 2×10^{-6} sec. Furthermore, streamers start from *both* electrodes, so that some ions move with, and some against, the field.

It is difficult to see how diffusion of vapor, in the ordinary sense, from the region of the electrodes can play the leading role in the production of streamers in sparks produced by short period circuits. That the diffusion is too slow is shown by the following consideration. Suppose the most favorable condition for rapid diffusion exists, *i.e.*, that all the material vaporized from the electrode during a half oscillation be concentrated in a small region at the electrode surface at the beginning of the half oscillation. The diffusion equations are similar to the equations for the flow of heat from an instantaneous point source (2, p. 149 ff). At a time t after the formation of the source, the concentration, C , at a distance, r , from the electrode, is,

$$C = Q/(2\sqrt{\pi Dt})^3 \cdot e^{-r^2/4Dt}, \quad (1)$$

where Q is the amount of vapor originally in the source, and D is the diffusion coefficient. The maximum concentration at a distance r from the electrode is attained in a time t_m , given by,

$$t_m = r^2/6D \quad (2)$$

and is,

$$C_m = 0.196Q/r^3. \quad (3)$$

The value of D for fairly heavy atoms at a temperature of 2000°K . may be taken to be about 4.5 (13). If the temperature in the gap be $10,000^\circ\text{K}$. (4), D should, from kinetic theory considerations ($D \propto \sqrt{T}$), be about 10.1, but might be as high as 22 ($D \propto T$). Using 10.1 for D , it is calculated that the maximum concentration at a point 1 mm. distant from the electrode is attained only after 1.7×10^{-4} sec. The concentrations at this point calculated in terms of the maximum concentration have the following values at the stated times.

$t(\text{sec.} \times 10^6)$	=	1	2	4	100
C/C_m	=	4×10^{-105}	10^{-31}	5×10^{-25}	0.29

The figures show that if the concentration at the point is to be detectable after say, 4×10^{-6} sec., the maximum concentration attained has to be many powers of 10; this is of course out of the question. Furthermore, even if D be taken to have the unreasonably large value of 300 in order to account for an appreciable concentration at 1 mm. from the electrode after 4×10^{-6} sec., the diffusion equations can hardly account for a comparable concentration at 3 mm. from the electrode after, say, 12×10^{-6} sec.

In view of the above considerations, it is highly probable that neither the motion of electrons or ions under the influence of the field, nor the diffusion of particles in the ordinary sense, can account for the characteristic features of the streamers produced in sparks with very small oscillation periods. The explanation must be sought in some other phenomenon. An interpretation of many of the observed features may be made on the basis of the existence of a pressure wave which moves out from the electrodes at each half oscillation. While reasonable inferences leading to the description of a possible transport mechanism may be drawn concerning the origin of the disturbances, such deductions are too speculative to justify detailed discussion in the present article. The exact nature of the transport mechanism in sparks requires further experiments for its elucidation.

Calculations from the previously given diffusion equations indicate that diffusion of material in the ordinary sense is sufficiently rapid to play a considerable part in the transport phenomena in sparks having a period of the order of 10^{-4} sec. Since the mechanism referred to above must also enter, however, the resulting phenomena are undoubtedly of a complex nature.

Whatever the nature of the transport mechanism may be, it involves collisional processes. Hence the rate of transport of an element along the discharge axis depends on the characteristics of the element, and on the characteristics of other particles present in the gap, *i.e.*, any damping of the translational motion of particles along the axis must depend on such factors as the number of elastic and inelastic collisions, the collisional cross section and hence the velocity, the ease of ionization, and the masses of the particles. It is undoubtedly on this basis that the results of Section 2 are to be explained.

4. Significance of the Data for Practical Analysis

Quantitative spectroscopic methods of analysis are based on the assumption that the intensity ratio of two lines due to the internal standard and investigated element is a constant function of the amounts of the elements present in the samples. It is clear from the data of Section 2 that when an image of the source is focused on the spectrograph slit, this ratio depends in general on the point in the source from which the examined light is radiated. Furthermore, the ratio for light radiated from a given point in the discharge may depend so strongly on the character of the extraneous material present in the samples as to make determinations based on it worthless as precision measurements (cf. Fig. 1). The variations that may occur in the ratio when no condensing lens is used, or when an image of the source is focused, say, on the collimator lens, reflect in addition any variations that may occur in the mechanism responsible for the release of material from the electrode, and so lie outside the scope of the present article. It is unquestionably important, however, that the operation of the various source mechanisms be kept as constant as possible, no matter what the optical set-up may be.

On the basis of the present results, the following precautions appear to be sufficient to minimize the effects of variations in the transport mechanism in the spark discharge: (a) addition to the sample of a "large" excess of some suitable salt (the spectroscopic buffer), and (b) the choice of an appropriate internal standard element.

(a) *The spectroscopic buffer.* For the present purpose the buffer should contain an element of low ionization potential. If such an element is present in sufficient amount, reasonable variations in the extraneous composition of the samples are not expected to materially alter the transport phenomena, since it was found that the relative distributions of intensity were independent of the amount of salt added within stated limits. The buffer should be capable of drying on the electrode in a closely adhering, uniform layer which is not flaked off by the action of the spark, and should not give rise to a complicated spectrum.

Organic salts are usually to be preferred to inorganic salts, since they form more suitable layers on the electrode. Sodium potassium tartrate has been found to be satisfactory, but cannot be used in analyses for sodium or potassium. Lithium tartrate has been used in such analyses, but it is possible that the precision of the determinations might be increased by the use of a buffer containing a more easily ionized element. The probable error was about 4% when lithium tartrate was used (6).

(b) *The choice of an internal standard element.* It is obviously desirable that the investigated and internal standard elements should behave in nearly the same way in the discharge. So far as the question of transport is concerned, they should have as nearly as possible the same ionization potential. Moreover, if the investigated element has an atomic mass of less than, say, 50, an internal standard element having as nearly as possible the same

mass should be chosen. With the heavier elements, however, the mass becomes unimportant.

For other reasons, the following characteristics are important in an internal standard element. It should give rise to suitable lines in convenient spectral regions, and should not interfere with the lines to be examined; the excitation potentials of the investigated element and internal standard lines examined should be as nearly as possible the same.

The relative importance of fulfilling any one of these different criteria depends on the characteristics of the investigated element; in many cases it may be necessary to partially disregard some of them in order to fulfil the most important. It may be pointed out that further experiments on the mechanism responsible for the release of material from the electrode may indicate other desirable qualities in both internal standard elements and spectroscopic buffers.

Acknowledgments

The authors are indebted to Dr. J. S. Foster for critical discussion of the data, and to the Rockefeller Foundation for financial assistance.

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NOTE ON THE ANALYSIS OF THE γ -RAYS OF RADIUM E¹BY J. A. GRAY² AND J. F. HINDS³

It is assumed that the energy in a beam of ionizing rays is proportional to the ratio of the ionization produced by the beam in a thin layer of gas, carbon dioxide in the present work, to the mass absorption coefficient of the rays in the gas. The relative numbers of particles (photons) in different beams can then be found by dividing relative energies by the energy of a single particle.

There is little doubt that the γ -rays of radium E form a continuous spectrum. The absorption curve of these γ -rays in aluminium has been obtained by the writers, who have shown also that, under the conditions of the experiment, this absorption is closely fitted by the equation

$$I = 26.3e^{-130m} + 32.5e^{-29m} + 13.9e^{-7.03m} + 7.7e^{-2.79m} + 19.6e^{-0.19m},$$

where I is the ionization produced by the rays in the ionization chamber, after the rays have passed through an aluminium sheet of mass m per sq. cm. This equation holds only up to a value of m of 2.45 gm. A separate experiment showed that the soft γ -rays of radium D, which are "L" rays from an element of atomic number 83, give under the same conditions an initial ionization of 2660.

The γ -rays of radium E were considered to consist of a number of component beams, of relative mass absorption coefficients 130, 29, 7.03, and the other values. The respective energies in electron volts of single photons in these components are 5800, 9700, 15,800, 21,600, and 63,000, these figures being obtained from the wave-lengths corresponding to the respective absorption coefficients.

Before the relative numbers of photons in the various components can be calculated, correction has to be made for the absorption of the rays in the air traversed by them before reaching the window of the ionization chamber, for absorption in this window, and for absorption in the gas filling the chamber. There is a further correction for the effect of the walls of the chamber, which were of steel. When all these corrections are made, the relative numbers of photons are 5 of 5800, 5.7 of 9700, 3.8 of 15,800, 2.1 of 21,600, and 20 of 60,000 volts and greater, respectively. For radium D soft γ -rays, the corresponding number is 630.

Previous experiments by the senior author showed that approximately 25% of radium D atoms emit a soft γ -ray, so that the effect of the γ -rays of radium E, as far as absorption in aluminium is concerned, is the same as if on disintegration 0.2% of the atoms emitted photons of energy 5800 volts, 0.23% photons of energy 9700 volts, 0.15% photons of energy 15,800 volts, 0.08% photons of energy 21,600 volts, and 0.8% photons of energy 60,000 volts and greater.

¹ Manuscript received February 21, 1938.

² Contribution from the Department of Physics, Queen's University, Kingston, Canada.

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⁴ Former holder of a bursary under the National Research Council of Canada; now at the California Institute of Technology.

If 40,000 volts is taken as midway between 21,600 and 60,000, then about 0.7% of the disintegrating atoms emit photons with energies less than 40,000 volts, and about 0.8%, photons with energies greater than this. There will be, of course, some atoms emitting photons with energies less than 5000 volts.

It is difficult to see how one can express the properties of the γ -rays of radium E in a more suitable manner. Analysis of the rays in the hardest component will be attempted in the near future.

NOTE ON A VACUUM TIGHT SEAL FOR ELECTRODES WITH HIGH INSULATION

The method described was used after it had been found that hard sealing wax plugs have a tendency to flow when subjected to pressure differences of the order of one atmosphere, particularly when the room temperature exceeds 70° F.

A permanent high insulation seal can easily be made from materials available in almost any workshop. The accompanying figure is drawn to scale, but the dimensions may be varied over a wide range to suit requirements.

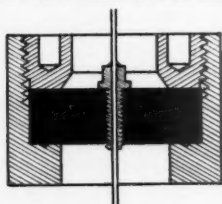


FIG. 1. Diagram of vacuum tight seal with high insulation.

An ebonite plug is made so as to fit freely in a brass tube, and is pressed against a shoulder by a threaded brass ring, which has two holes drilled in it to take a face pin wrench. The shoulder has a small annular V-shaped groove cut in it to facilitate making the joint.

A standard brass screw, faced smooth under the head and with two small flats filed at right angles to the slot, is drilled to take the electrode which is soldered at the outer end. The screw is tightened in the plug by using a wrench on the flats, after which the faces of the ebonite are carefully scraped. It is important that these surfaces should not be touched with the fingers after this operation. The plug is immersed in melted paraffin for a sufficient length of time to heat it so that the wax runs off freely when it is removed. A thin coating is desirable, and is all that is necessary to protect the ebonite from the action of the atmosphere. Rapid rotation of the plug is effective in removing excess wax.

After cooling, the plug is inserted in the tube and the ring screwed down. No gasket is necessary. Joints of this type have been in use for years and have given complete satisfaction.

Note by J. A. Gray

Other people may know of the insulator, the preparation of which has been described above by Mr. Bradfield, but a great many do not. We are using it in electrometers, ionization amplifiers, and ordinary electroscopes, and find it very satisfactory indeed; so much so, that we do not bother about other insulators. The only disadvantage that we have noticed so far about its use, is that it is not suitable in the presence of methyl iodide.

R. D. BRADFIELD.

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THE STRUCTURE OF DEXTRAN

NOTE ON "THE STRUCTURE OF DEXTRAN SYNTHESIZED BY THE ACTION OF *LEUCONOSTOC MESAENTERIOIDES* ON SUCROSE"
BY FOWLER, BUCKLAND, BRAUNS, AND HIBBERT¹

By F. E. BRAUNS²

Since a preliminary experiment on the alcoholysis of methylated dextran revealed that three different methylated methyl glucosides are obtained, two principal requirements must be fulfilled in order to determine the structure of dextran: first, the dextran must be methylated completely, as on hydrolysis of an incompletely methylated dextran more lower-methylated glucoses are obtained; second, the different methylated glucosides have to be separated quantitatively in order to determine the exact ratio of dimethyl, trimethyl, and tetramethyl methyl glucosides. Such a quantitative separation cannot be carried out by fractional distillation, as has been confirmed by other carbohydrate chemists.

Such a quantitative separation has *not* been accomplished in the work described, and therefore it is not possible to decide definitely the structure of dextran. In Experiment I, in which 7.5 gm. of the original hydrolysis products was separated, the loss during the separation was 18.4%, and, when this loss is allocated *pro rata* to these methyl glucoside fractions, the corrected yields are 1.70, 4.34, and 1.47** gm. for dimethyl, trimethyl, and tetramethyl methyl glucosides, respectively, as given in Table I of the paper. These corrected values, however, are not used in the calculation of the yields of methyl glucosides in the various ratios. If the corrected values as given in percentage in Column 3 of Table A are used, the actual yields of the three glucosides, compared with those calculated for a ratio of 1 : 3 : 1, would be 120.5, 96.4, and 92.5%, respectively, instead of 99.3, 78.3, and 75.5. This experiment, therefore, actually indicates that dextran is not a 1 : 3 : 1 polymer. If, however, the loss is allocated to the trimethyl and tetramethyl methyl glucosides only, on the assumption that the loss first occurred during the separation of these two, as indicated in Experiment II, the corrected values as given in Column 4 are obtained; these agree with the

¹ Original manuscript received January 5, 1938.

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* The manuscript of this paper (published November, 1937, in the *Canadian Journal of Research*, B, 15, 486-497.) was sent to me by Dr. Hibbert for approval. Since it did not take into consideration the loss of material in the separation, it was returned with the notations made herein, but no major change was made, aside from the insertion in Table I of the corrected values, which were not used in the calculations.

** 1.17 as given in Table I should be 1.47.

TABLE A
YIELDS OF METHYL GLUCOSIDES

Methyl glucoside isolated	Yield from 7.5 gm. of hydrolyzed methylated dextran				Yield from 4.55 gm. hydrolyzed methylated dextran				Theoretical yield of dimethyl, trimethyl, and tetramethyl methyl glucosides in %, on the basis of the ratios shown			
	Gm.	%	Per cent corrected for the loss according to		Gm.	%	Per cent corrected for the loss	1 : 1 : 1	1 : 2 : 1	1 : 3 : 1	1 : 4 : 1	
			Hibbert†	Brauns††								
Dimethyl methyl glucoside Mol. wt. 222.15	1.40	18.7	22.67	18.7	0.69**	15.2	15.2	31.4	23.5	18.8	15.7	
Trimethyl methyl glucoside Mol. wt. 236.16	3.52*	46.9	57.87	60.6	2.35	51.7	67.3	33.3	50.0	60.0	66.6	
Tetramethyl methyl glucoside Mol. wt. 250.18	1.20	16.0	19.6	20.7	0.61	13.4	17.5	35.3	26.5	21.2	17.7	
Total yield	6.12	81.6	100.14	100.0	3.65	80.3	100.0					
Loss during separation		18.4				19.7						

* Does not contain the 0.05 gr. of Frac. Vc. since this residue was not crystalline and was not analyzed.

** Value taken from Miss Fowler's thesis.

† Allocated to all three glucosides.

†† Allocated to trimethyl and tetramethyl glucosides.

values for a 1 : 3 : 1 polymer. For a ratio of 1 : 2 : 1, the yields actually found, calculated as percentage of the theoretical values, are 79.5, 94.0, and 60.3% (as given in Column 6 of Table I of the paper); whereas the corresponding corrected yields are 96.6, 115.7, and 73.8%.

In the second experiment, in which 4.55 gm. of hydrolyzed methylated dextran is used for a quantitative separation, the refractive index of 1.455 for the mixture of tetramethyl and trimethyl methyl glucosides is applied for the determination of the ratio of the amounts of each present, which is thus found to be 25% tetramethyl to 75% trimethyl methyl glucoside. A glance at the refractive indices given in the literature for the various methylated methyl glucosides prepared by different investigators, shows that the refractive indices, even of the same methyl methyl glucoside, vary over a comparatively wide range, so that they cannot be used as a basis for quantitative analytical calculations. On the other hand, if average refractive indices are used, it can be seen that the refractive indices for mixtures are too close together to permit their being used as a basis for the calculation of the amounts of two different methyl methyl glucosides in a mixture of the two. If for a mixture with $n_D = 1.455$, the calculated ratio is 25% tetramethyl to 75% trimethyl methyl glucoside, the ratio for a mixture with $n_D = 1.454$ would be 33.3 to 66.6%, and for one with $n_D = 1.456$, it would be 16.6 to 84.4%. Nor do the yields of the isolated dimethyl, trimethyl, and tetramethyl methyl glucosides in the second experiment justify the statement that dextran is a 1 : 3 : 1 polymer. In the fifth and sixth columns of Table A, the yields of dimethyl, trimethyl, and tetramethyl methyl glucosides actually found are given. After removal of the dimethyl methyl glucoside (0.69 gm.), a yield of 3.85 gm. of a mixture of trimethyl and tetramethyl methyl glucosides is obtained; this shows that the loss of 19.7% of the total yield at the end of the separation occurred during the fractionation of the tetramethyl from the trimethyl methyl glucoside. When this loss is allocated in a ratio of 51.7 to 13.4% to the trimethyl and tetramethyl methyl glucoside fractions, respectively, their yields become 67.3 and 17.5%. The total yields of dimethyl, trimethyl, and tetramethyl methyl glucosides in the second experiment are, therefore, 15.2, 67.3, and 17.5% (Column 7, Table A); these figures agree with those for a 1 : 4 : 1 polymer (Column 11, Table A).

This detailed statement clearly indicates that it cannot be decided definitely whether dextran is a 1 : 2 : 1, 1 : 3 : 1, or 1 : 4 : 1 polymer on the basis of the information contained in the paper under discussion. This question can be solved only by a quantitative separation of the methylated methyl glucosides obtained on alcoholysis of fully methylated dextran.

ANOLOBINE, AN ALKALOID FROM *ASIMINA TRILOBA* DUNAL¹

BY RICHARD H. F. MANSKE²

Abstract

An alkaloid, for which the name anolobine is now suggested, has been isolated from the bark of *Asimina triloba* Dunal, the North American papaw. It is best represented by $C_{17}H_{17}O_3N$ and contains one phenolic hydroxyl group. Gaebel's reaction indicates a methylenedioxy group. The nitrogen is secondary. Exhaustive methylation and oxidation of the final vinyl compound has yielded 4-methoxyphthalic acid, and this observation together with a knowledge of analogous alkaloids from related plants has made it possible to designate a highly plausible structure for anolobine. A glycoside was found in the leaves although it does not appear to be present in the bark.

The alkaloids of the Natural Family, Anonaceae, have received only scant attention. *A priori*, a close chemical correspondence between alkaloids of this family and those of Menispermaceae and Lauraceae is to be suspected. Botanical classification includes the three families in the Natural Order Ranunculales.

The family, of which *Asimina triloba* Dunal is the only Canadian representative, is widely distributed throughout the tropics. The genus, *Anona*, regarded by some botanists as synonymous with *Asimina*, is represented in the tropical Americas by a number of species, some of which have yielded alkaloids, though so far of undetermined structure (3, 4, 6). It was therefore of interest to investigate the plant named in the title. It grows as a shrub or a small tree, and the pulp of the fruit, known as the North American papaw, is edible when fully mature.

The examination of the leaves failed to reveal more than doubtful traces of alkaloids. The bark contained about 0.05% of total alkaloid whether it was collected in summer or in winter. Though minute amounts of other bases appear to be present, the only alkaloid thus far obtained crystalline constitutes nearly 90% of the total. This alkaloid, for which the name anolobine is now proposed, is best represented by $C_{17}H_{17}O_3N$. Its monophenolic nature is proved by the preparation from it of a non-phenolic monomethyl ether, $C_{18}H_{19}O_3N$. The remaining two oxygens are almost certainly present in a methylenedioxy group. It readily gives a precipitate of the red phloroglucide in Gaebel's test, although the formal proof of the presence of such a group is still lacking. The nitrogen atom is secondary. The phenolic base, as well as its methyl ether, yields a water insoluble nitrosamine, which is soluble in ether and gives Liebermann's nitroso reaction with great brilliance.

Methylation of the methyl ether yields a mixture containing a moderate quantity of the quaternary iodide, and this on decomposition with strong

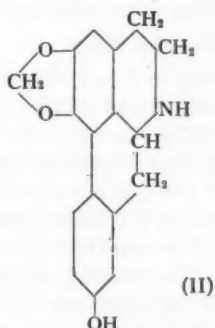
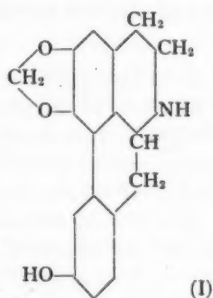
¹ Manuscript received January 25, 1938.

Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

Chemist, National Research Laboratories, Ottawa.

alkali yields an optically inactive methine, $C_{20}H_{23}O_3N$. Further degradation of the latter via its methiodide yields a hydrocarbon which is at least partly polymerized. On the assumption that anolobine is an aporphine alkaloid, this hydrocarbon on oxidation should yield a methoxy-methylene-dioxy-phenanthrene carboxylic acid. The main product obtained however proved to be identical with 4-methoxyphthalic acid. There was obtained only a minute amount of an acid which might have been a derivative of phenanthrene.

This observation serves to locate the phenolic hydroxyl as in Formulas (I) or (II), the process of oxidation having destroyed the benzene nucleus carrying the methylenedioxy group. The first of these is excluded because the methine



of anolobine methyl ether is certainly not identical with that of laureline, which on the basis of degradative experiments (1) and a synthesis (5) is the ON-dimethyl derivative of (I). The allocation of the methylenedioxy group to the position assigned to it is based on analogy with its similar position in virtually all known aporphine alkaloids. Formula (II) is therefore ascribable to anolobine with a considerable degree of certainty, although a rigorous proof by synthesis is desirable. The author takes this opportunity to invite anyone interested to undertake such a problem.

Anolobine

Experimental

The dried bark of *Asimina triloba* Dunal, collected in southwestern Ontario, was extracted with methanol and the solvent removed from the extract. The addition of water and enough hydrochloric acid to yield a solution acid to Congo red caused the separation of a rather low melting fat, which was separated only with difficulty. The clear aqueous solution which was ultimately obtained was first extracted several times with ether, then basified with excess ammonia, and extracted with ether again. In some experiments the second extraction was carried out with chloroform, but in either case the large amount of inorganic precipitate made the separation a tedious one.

Removal of the solvent from the extract yielded a residue that crystallized readily in contact with methanol when crystals for inoculation were available. Owing to its sparing solubility in methanol, the crude anolobine as thus

obtained could be readily purified by washing with this solvent in which the colored impurities were to a large extent soluble. Repeated recrystallization from chloroform-methanol, or purification via the sparingly soluble hydrochloride, yielded brilliant colorless prisms, which when placed in a bath at 245° C.* melted to a dark tar at 262° C., some darkening taking place about 10° lower. Anolobine dissolves in sulphuric acid to form an intense green solution, which on gentle warming becomes olive in color; on further heating, the color changes to brown. Nitric acid yields a pink solution which becomes orange colored on warming. Calcd. for $C_{17}H_{17}O_3N$: C, 72.08; H, 6.01; N, 4.95%. Found: C, 72.77; H, 5.54; N, 5.11%.

Anolobine is laevorotatory, giving a value, $[\alpha]_D^{27} = -22.5^\circ$ in the solvent made by mixing equal volumes of chloroform and absolute methanol ($c = 0.4$).

Anolobine O-Methyl Ether

A suspension of 2 gm. of anolobine in 50 cc. of chloroform containing some methanol was treated with an ethereal solution of diazomethane. The alkaloid slowly dissolved as the liberation of nitrogen proceeded. After 24 hr. the alkaloid had dissolved and the reaction appeared to be completed. Removal of the organic solvents and addition of dilute hydrochloric acid yielded a sparingly soluble hydrochloride. The filtered solution of the latter in hot water was basified with excess alkali, and the liberated base extracted with ether. The washed extract was evaporated to a small volume. The methyl ether of anolobine crystallized readily. It was recrystallized first from dry ether, in which it is only sparingly soluble, and then from methanol-ether. It was obtained in colorless fine needles, which melt at 97° C. and in contact with air gradually develop a slight greenish color. It dissolves in cold sulphuric acid to form a colorless solution which rapidly becomes pink. Gentle heating deepens this color, which on progressive heating becomes olive and then dark olive blue. Dilution with water yields a Prussian blue solution.

The methyl ether, like the parent alkaloid, yields an ether soluble precipitate when its solution in dilute acid is treated with sodium nitrite. The precipitate yields an intense blue color in Liebermann's test for nitroso compounds. Calcd. for $C_{18}H_{19}O_3N$: C, 72.73; H, 6.40; N, 4.71; 1 OMe, 10.44%; M.W. 297. Found: C, 71.31; H, 5.97; N, 4.86; OMe, 10.29%; M.W. 302. $[\alpha]_D^{27} = -27.9^\circ$ ($c = 0.4$ in absolute methanol).

Anolobine O-Methyl Ether Methine

The fact that anolobine is a secondary base is satisfactorily demonstrated by the mixed nature of the product obtained when the methyl ether is treated with methyl iodide. A solution of the alkaloid in methanol on treatment with methyl iodide yielded an almost instantaneous precipitate of the crystalline, though not homogenous, addition product. The organic solvents used were boiled off, and the residue was dissolved in a large volume of boiling water. The filtered solution was rapidly cooled and basified with an excess of potassium hydroxide. The mixture of secondary and tertiary bases thus liberated was extracted with ether and treated again with methyl iodide. The quaternary

* Melting points are corrected.

methiodide crystallized for the greater part from the cooled solution, and was decomposed by heating for 24 hr. on a steam bath in strongly alkaline solution. The liberated methine base was extracted from the cooled solution, and a further small quantity obtained by heating the aqueous solution again.

The methine as thus obtained crystallized readily when its ether solution was evaporated. It was recrystallized from dry ether and obtained in long colorless needles that melt at 99° C. In admixture with anolobine O-methyl ether, this substance was completely liquid at 84° C. In absolute methanol it was optically inactive ($c = 0.4$). Calcd. for $C_{20}H_{23}O_3N$: C, 73.85; H, 7.08; N, 4.31; 1OMe, 9.54%. Found: C, 74.24; H, 6.51; N, 4.57; OMe, 9.48%.

4-Methoxyphthalic Acid

The above-described methine on treatment with methyl iodide in methanol yielded a very sparingly soluble methiodide. This was decomposed by heating on the steam bath with an excess of aqueous potassium hydroxide. After an interval of 24 hr. the solution was cooled and the liberated hydrocarbon extracted with ether. This process was repeated until no more hydrocarbon was obtained, and until the methiodide had virtually disappeared. The hydrocarbon was definitely crystalline although a portion of it appeared to be polymeric. It was oxidized with potassium permanganate in acetone solution containing a little water until the permanganate color was permanent for five to six hours. Water was then added and the acetone boiled off, and the precipitate of manganese dioxide dissolved in a stream of sulphur dioxide. The acidified solution (hydrochloric acid) was thoroughly extracted with ether. Removal of the solvent yielded a residue which largely crystallized although it was evidently a mixture. The greater portion of it was very soluble in water, and this fraction was extracted from a very small amount of a sparingly soluble acid. The latter was dissolved in dilute sodium bicarbonate solution and the filtrate acidified. In the course of several days the sparingly soluble acid separated in slender prisms that melted at 204° C. After recrystallization from methanol, it melted at 207° C. The amount of this acid was insufficient for an analysis, in spite of the fact that a total of 3.7 gm. of anolobine was used in these experiments. It seems highly probable, however, that it is 3 : 4 methylenedioxy-7-methoxy-phenanthrene-1-carboxylic acid.

The readily soluble portion of the oxidation product was distilled up to 115° C. at 1 mm. and the distillate redistilled at 110° at 1 mm. It then melted at 95° C. After one recrystallization from benzene-petroleum ether it melted at 96° C., and in admixture with an authentic specimen of 4-methoxyphthalic anhydride melting at 98° C. (2) it melted at 97° to 98° C.

The methyl-imide was distilled *in vacuo* and after one recrystallization from methanol melted at 158° C. either alone or in admixture with a synthetic specimen. The ethyl-imide was prepared and distilled in the same manner. It was recrystallized from petroleum ether, and either alone or admixed with an authentic specimen it melted at 79° C. Calcd. for $C_{11}H_{11}O_3N$: N, 6.83%. Found in synthetic specimen: N, 6.69%.

Isolation of a Glycoside (?)

The dried leaves (1910 gm.) collected in August were examined for alkaloids. The clear aqueous solution obtained from the methanolic extract was allowed to remain for several days, during which time a sparingly soluble substance crystallized. This was filtered off, washed, and dried. It was dissolved in a small volume of methanol in which it is readily soluble, and the hot filtered solution cautiously treated with hot water. Pale yellow fine needles in stellate aggregates were thus obtained. After filtering, washing, and drying, this substance, which is probably a glycoside, melted at $186^{\circ}\text{C}.$, sintering slightly at 184° to $185^{\circ}\text{C}.$ It does not contain methoxyl, and the analyses are in agreement with the dihydrate of $\text{C}_{21}\text{H}_{24}\text{O}_{11}$, although $\text{C}_{21}\text{H}_{22}\text{O}_{11}$ is not entirely excluded and is more probable on structural grounds. Calcd. for $\text{C}_{21}\text{H}_{24}\text{O}_{11} \cdot 2\text{H}_2\text{O}$: C, 51.64; H, 5.74%. Found: C, 51.04; H, 5.51%.

Only the merest trace of alkaloid was found in the leaves, and its presence here may be attributable to admixture of a small amount of bark from the younger twigs which was peeled off as the leaves were stripped from the tree. The glycoside was not found in the bark.

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THE ALKALOIDS OF FUMARIACEOUS PLANTS

XVI. SOME MISCELLANEOUS OBSERVATIONS¹

BY RICHARD H. F. MANSKE²

Abstract

Eleven new alkaloids are described. They have been obtained from mother liquors from plants whose investigation has already been reported. The present record deals with *Dicentra cucullaria* (three)*, *D. eximia* (four), *Corydalis aurea* (seven), and *C. sempervirens* (two). For convenient reference all new alkaloids in this series of communications have been given a number following the letter F, and a table is included in which the salient features are summarized.

Alkaloid F 23 (formerly - η) has now been obtained from *C. sempervirens*. It is shown to be the optical antipode of *d*-adlumine, and a mixture of the two has yielded the *dl*-form.

The thoroughness with which the alkaloids of a particular plant may be isolated and characterized depends, in addition to the amount of material available, on the number of alkaloids present and on their crystallizability either as free bases or as simple derivatives. With mixtures of unknown constituents, crystallization of an individual is frequently a matter of chance, dependent, among other factors, on time and on the absolute amount. It may be permissible, therefore, to reiterate that the investigations which the author has recorded in this field from time to time cannot be regarded as exhaustive. In general, at the time of publication, small amounts of uncrystallized mother liquors remained, and the further examination of these seemed unprofitable until more material became available, or until spontaneous crystallization took place. The nucleus required to induce crystallization of a particular fraction of some of the mother liquors was obtained from a new and higher yielding source.

In the following summary the names of the alkaloids thus far isolated are shown after the name of the plant from which they were obtained. Those in parentheses have been previously reported and are included to complete the record.

Dicentra cucullaria,—(protopine, cryptopine, α -allocryptopine, bicuculline, corlumine), cularine, cularidine, ochotensine.

Corydalis sempervirens,—(protopine, cryptopine, bicuculline, capnoidine), F 20, F 23.

Dicentra eximia,—(protopine, dicentrine, glaucine, corydine, glaucentrine), cularine, F 21, F 29, F 30.

Corydalis aurea,—(protopine, bicuculline, capaurine, capauridine, corydaline, *el*-tetrahydropalmatine, corypalline), *dl*-tetrahydropalmatine, α -allocryptopine, aurotensine, cordrastine, F 24, F 27, F 28.

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* Number of hitherto unreported alkaloids.

At the outset of these investigations it was not realized that so many new alkaloids would be found in the Fumariaceae family. When a definite name could not be coined on one basis or another, resort was had to the letters of the Greek alphabet to designate new or supposedly new alkaloids. The limitations of such a system have now become apparent. More than 30 alkaloids for which no name has yet been evolved and which appear to be new have already been isolated. It is therefore proposed to allocate to each of these alkaloids a number following the letter F (from Fumariaceae). Furthermore, in order to systematize the record, all alkaloids found by the author whether they have already been given a name or a Greek letter are reclassified. Table I, including 11 hitherto unreported alkaloids, is a review as well as a summary.

TABLE I

No.	Name or designation	M.p., °C.*	Formula	Functional groups	References
F 1	Bicuculline (α)	177	$C_{20}H_{17}O_4N$	$2 O_2CH_3$	2, 3, 4, 6, 8, 12, 13, 15
F 2	<i>d</i> -Adlumine	180	$C_{21}H_{19}O_4N$	O_2CH_3 ; $2 OMe$	4, 5
F 3	Adlumidine	235	$C_{19}H_{19}O_4N$	$2 O_2CH_3(?)$	4
F 4	Capnoidine	238	$C_{19}H_{19}O_4N$	$2 O_2CH_3(?)$	6, 12
F 5	Glaucentrine (δ)	148	$C_{20}H_{20}O_4N$	$3 OMe$; OH	7, 9, 10
F 6	Capaurine	164	$C_{21}H_{17}O_4N$	$4 OMe$; OH	8
F 7	Capauridine	208	$C_{21}H_{17}O_4N$	$4 OMe$; OH	8
F 8	Corypalline	164	$C_{21}H_{19}O_4N$	OMe ; OH	14
F 9	Cularine	115	$C_{20}H_{19}O_4N$	$3 OMe$	**
F 10	Cularidine	157	$C_{19}H_{19}O_4N$	$2 OMe$; OH	**
F 11	Corlumine	159	$C_{21}H_{19}O_4N$	O_2CH_3 ; $2 OMe$	11, 12, 13
F 12	Corlumidine	236	$C_{20}H_{19}O_4N$	O_2CH_3 ; OMe ; OH	11, 12, 14
F 13	θ	183	$C_{18}H_{19}O_4N$	O_2CH_3 ; OMe ; OH	12, 13
F 14	κ	198	$C_{20}H_{19}O_4N$	$O_2CH_3(?)$	13
F 15	λ	212	$C_{19}H_{19}O_4N$	$O_2CH_3(?)$; $2 OMe$	13
F 16	μ	236	$C_{18}H_{17}O_4N$	$O_2CH_3(?)$; $2 OMe$	13
F 17	Ochotensine (ι)	248	$C_{21}H_{21}O_4N$	$O_2CH_3(?)$; OMe ; OH	13
F 18	Aurotensine	128	$C_{19}H_{19}O_4N$	$2 OMe$; $2 OH$	**
F 19	Cordrastine	196	$C_{20}H_{20}O_4N$	$4 OMe$	**
F 20		221	$C_{18}H_{19}O_4N$		**
F 21		80	$C_{20}H_{20}O_4N$	$4 OMe$	**
F 22		238	$C_{27}H_{40}O_{10}N_2$	$3 OMe$	1
F 23	<i>l</i> -Adlumine η	180	$C_{21}H_{19}O_4N$	O_2CH_3 ; $2 OMe$	12
F 24		138	$C_{19}H_{19}O_4N$	$3 OMe$; OH	**
F 25	ν	230	$C_{19}H_{17}O_4N$	$O_2CH_3(?)$; NMe	15
F 26	Cryptocavine	223	$C_{20}H_{20}O_4N$	O_2CH_3 ; $2 OMe$	15
F 27		148	$C_{21}H_{20}O_4N$	$4 OMe$	**
F 28		135	$C_{17}H_{19}O_4N$	$2 OMe$; OH	**
F 29		262	$C_{19}H_{19}O_4N$	$2 OMe$; $2 OH$	**
F 30		102	$C_{19}H_{19}O_4N$	$3 OMe$	**
F 31		216	$C_{18}H_{19}O_4N$	$O_2CH_3(?)$	15

* Melting points are corrected.

** Alkaloids that are discussed in this paper.

Special interest attaches to alkaloid F 23, formerly alkaloid- η (12). Although it has been available in only very small amounts, it is now shown to be the optical antipode of *d*-adlumine (15) from *Adlumia fungosa*. With *d*-adlumine it yields *dl*-adlumine which crystallizes in stout prisms quite distinct from either of its parents. The constitution of *l*-adlumine has also been formally

demonstrated by hydrolytic oxidation. The acidic and basic fragments thus obtained were characterized in each case by a Cannizzaro rearrangement which yielded 3:4-methylene-dioxy-phthalide and 1-keto-2-methyl-6:7-dimethoxy-tetrahydro-isoquinoline respectively.

Experimental

In the following résumé the known alkaloids which have not previously been described as occurring in the plants mentioned are only briefly recorded. The new alkaloids and their isolation are described in greater detail. The nomenclature of the various fractions is that previously recorded (4).

α-Allocryptopine and Corlumine from D. cucullaria

The mother liquors which had accumulated as the result of isolating the known alkaloids from 93 kg. of plant material (tubers and aerial portion) were combined and reworked (4). The protopine fraction (BS) finally crystallized for the greater part, and when this alkaloid was recrystallized from chloroform-methanol it melted at 160° C.* This melting point was not depressed when the alkaloid was admixed with an authentic specimen of *α*-allocryptopine.

The mother liquor from which the bicuculline had been crystallized was clarified in methanol with charcoal and seeded with a crystal of corlumine. Crystallization was immediate, and recrystallization yielded colorless fine needles, melting alone or admixed with an authentic specimen at 159° C. The total yield was 0.04%.

The final mother liquor on long standing deposited a small amount of corlumine and with it a small amount (0.1 gm.) of a very sparingly soluble alkaloid. Hot methanol removed the former, and the latter was recrystallized from hot chloroform, in which it is only sparingly soluble. This proved to be identical with F 17 (alkaloid-*ι*), first isolated from *C. sibirica* (13). Its relative abundance in *C. ochotensis*, a plant now under investigation, suggests the name *ochotensine*.

Glaucentrine

This is the name now given to an alkaloid first isolated from *D. eximia* and later from *D. formosa* and *D. oregana*. It was obtained crystalline only as the hydrochloride and referred to as alkaloid-*δ* (7, 9, 10). The free base has now been obtained crystalline. For this purpose the purified hydrochloride was dissolved in water, treated with aqueous sodium bicarbonate, and the liberated base extracted with purified ether. The washed and dried solution (sodium sulphate) was evaporated to a small volume. The alkaloid separated for the greater part as a viscous resin. Dry methanol was added until the solution just became homogeneous, and the solution was placed in the ice chest. The base crystallized after some time. It was filtered off, washed first with ether containing a little methanol, and then with dry ether in which it is only sparingly soluble. Colorless fine prisms melting at 148° C.

* All melting points are corrected.

were thus obtained. Analyses† indicate the formula $C_{20}H_{23}O_4N$. Calcd.: C, 70.38; H, 6.75; N, 4.00%. Found: C, 70.02; H, 6.84; N, 4.05%.

The previous formula (9), $C_{21}H_{25}O_5N$, is erroneous, although the presence of three methoxyl groups is clearly indicated. The hydrochloride was found to contain 23.84% methoxyl; $C_{20}H_{23}O_4N \cdot HCl$ requires 24.64%. Final evidence for the correctness of the new formula was obtained when it was observed that the fully methylated alkaloid (diazomethane) is identical with *d*-glaucine. Glaucetrine therefore is *O*-desmethylglaucine. Work is in progress with the object of determining which of the four possible formulas is correct.

Alkaloid F 29

The fractions (BSE and EES) from *D. eximia*, which yielded glaucentrine, contained another alkaloid that crystallized with great facility in one particular experiment, and its subsequent isolation from the mother liquor was readily accomplished. The phenolic bases regenerated from the mother liquors obtained in the isolation of glaucentrine hydrochloride crystallized readily in contact with methanol. The base is virtually insoluble even in hot methanol, and was recrystallized from much boiling chloroform, and more conveniently from hot dioxane and methanol. It was then obtained in brilliant colorless prisms which began to darken at 250° to 255° C. and melted to an orange colored liquid at 262° C. The yield was 0.007%. Analysis indicate two methoxyl groups in $C_{19}H_{21}O_4N$. Calcd.: C, 69.74; H, 6.40; N, 4.28; 2OMe, 18.89%. Found: C, 69.67; H, 6.49; N, 4.65; OMe, 18.83%.

The presence of two hydroxyl groups in this alkaloid is confirmed by the preparation of its dimethyl ether. For this purpose an ethereal solution of diazomethane was added to a solution of the base in dioxane. After 24 hr. the non-phenolic base was purified as the hydrochloride, which is only sparingly soluble in cold water. This salt melts to an orange colored liquid at 236° to 237° C. The free base prepared from the hydrochloride was recrystallized from hot methanol (moderately soluble) and washed with ether in which it is sparingly soluble. Large stout prisms melting at 177° C. were thus obtained. Dissolved in sulphuric acid, the crystals yielded a colorless solution which on heating exhibited the following color changes—dirty olive, blue with a pink cast, and finally deep blue. Calcd. for $C_{21}H_{25}O_4N$; C, 70.99; H, 7.04; 4OMe, 34.93%. Found: C, 71.08; H, 7.18; OMe, 34.72%.

The diethyl ether was prepared in the same manner. The hydrochloride is readily soluble in dioxane and was purified by recrystallization from water. The free base was recrystallized from ether-petroleum-ether; the colorless prisms thus obtained melt at 131° C. Calcd. for $C_{23}H_{29}O_4N$; 2OMe and 2OEt as 4OMe, 32.38%. Found: OMe and OEt as OMe, 31.93%.

Corydalis aurea

To date, a total of 49 kg. of this plant has been worked over, and, aside from a large supply of mother liquors which have yielded no crystals, 14 alkaloids

† Analyses are the means of satisfactory duplicates.

have already been isolated. The recent discovery of corypalline (14) in the seeds is an indication that the genus *Corydalis* differs chemically from *Papaver* in that the seeds of the latter are devoid of alkaloids. The large number of alkaloids makes any exact description of their isolation difficult.

The crude tetrahydropalmatine obtained from fraction (BC) was recrystallized as the hydrochloride; a small amount of a very sparingly soluble salt was obtained. The latter salt was recrystallized several times from much boiling water, and it then consisted of pale yellow fine needles. The free base was generated from this by adding ammonia to a rapidly cooled aqueous solution of the salt. The caseous precipitate readily became granular as the base crystallized. It was recrystallized from methanol and obtained in colorless elongated plates which melt at 148° C. This is not identical with *dl*-tetrahydropalmatine described below. Its non-identity with several new alkaloids, all of approximately the same melting point and empirical composition, which have been isolated from other sources, does not help to elucidate its nature. It is F 27 and is best represented by $C_{21}H_{25}O_4N$, containing four methoxyl groups. Calcd.: C, 70.98; H, 7.04; N, 3.94; 4OMe, 34.93%. Found: C, 71.05; H, 7.00; N, 3.99; OMe, 34.84%.

Since the author's first paper on *C. aurea* (8), Eppson (16) has investigated the same plant. His C_1 , m.p. 138° to 140° C., is almost certainly *l*-tetrahydropalmatine, in that the optical rotation and the methoxyl content are approximately correct. C_2 is probably identical with the author's F 27, although its identity with the alkaloid of *C. ambigua* of the same melting point is less certain.

The author has isolated from other sources at least one more alkaloid of approximately the same composition and melting point. Eppson's D_2 is almost certainly a slightly impure specimen of the low melting form of protopine.

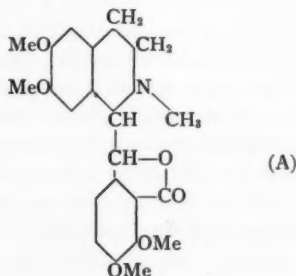
The mother liquor from which the *l*-tetrahydropalmatine had been crystallized was converted to hydrochloride. On cooling, a mixture of two hydrochlorides separated—fine needles, readily soluble on warming, and stout, highly refracting polyhedra only slowly soluble. The two were separated by gently warming a suspension of them in water. The filtrate redeposited the fine needles. Each fraction was recrystallized. The stout polyhedra yielded *d*-corydaline, and the fine needles on appropriate treatment yielded a base which was recrystallized from methanol; it then melted at 152° C. In admixture with a specimen of *dl*-tetrahydropalmatine, prepared from *l*-tetrahydropalmatine by oxidation with iodine and reduction of the palmatine iodide thus formed with zinc and hydrochloric acid, there was no depression in melting point. Calcd. for: $C_{21}H_{25}O_4N$; C, 70.98; H, 7.04; N, 3.94; 4OMe, 34.93%. Found: C, 71.16; H, 7.07; N, 4.31; OMe, 34.99%.

Aurotensine is the name now given to an alkaloid which has been isolated not only from *C. aurea*, but also from *C. ochotensis* and *Fumaria officinalis*. Furthermore, it is of interest to note that its dimethyl ether is the main alkaloid found in another species; namely, *C. caseana*, the investigation of which will be recorded shortly. It occurs in fractions (BSE and EES), from

which it is conveniently isolated as the sparingly soluble hydrochloride. The free base was regenerated from the hydrochloride by cautiously treating a rapidly cooled aqueous solution with dilute ammonia. It was recrystallized by adding a little water to a concentrated methanolic solution. Colorless, flat rhombic plates were thus obtained. It melts at $128^{\circ}\text{C}.$, losing water of crystallization at the same time. Calcd. for $\text{C}_{19}\text{H}_{21}\text{O}_4\text{N}$: C, 69.74; H, 6.40; N, 4.28; 2OMe, 18.89%. Found: C, 68.51; H, 6.62; N, 4.29; OMe, 19.61%. This formula is confirmed by the analysis of the dimethyl ether, which will be described in another communication.

Cordrastine

The existence of hydrastine, adlumine, and corlumine, and of bicuculline in nature renders the existence of the tetramethoxy analogue highly probable. On one occasion the protopine fraction from *C. aurea* was contaminated with a small amount of a second base which was separated from the former as its readily soluble hydrobromide. The regenerated base was recrystallized from chloroform-methanol, and then consisted of colorless fine needles which melted with previous darkening at $196^{\circ}\text{C}.$ Though the formal proof of its structure is lacking, this alkaloid, for which the name *cordrastine* is now proposed, is almost certainly represented by Formula A. A trace of this base, when heated with manganese dioxide in dilute sulphuric acid, yielded a strongly fluorescent solution, a behavior reminiscent of that of other phthalide-isoquinoline alkaloids. The analytical figures are in excellent agreement with those required by theory, although sufficient material for only one set of analyses was available. Calcd. for $\text{C}_{22}\text{H}_{25}\text{O}_6\text{N}$: C, 66.17; H, 6.27; N, 3.51; 4OMe, 30.08%. Found, C, 66.41; H, 6.32; N, 3.43; OMe, 29.37%.



Alkaloid F 24

The ether extract (EEC) from a large lot of *C. aurea* in contact with methanol deposited a mixture of bases, the more soluble of which was repeatedly recrystallized from hot methanol. It was ultimately obtained in colorless fine prisms that melted at $138^{\circ}\text{C}.$ Found: C, 69.65; H, 7.05; N, 4.41; OMe, 27.33%. Calcd. for $\text{C}_{19}\text{H}_{23}\text{O}_4\text{N}$: C, 69.72; H, 7.03; N, 4.28; 3OMe, 28.44%.

Alkaloid F 28

The fraction (ES) was freed of non-phenolic alkaloids, and the phenolic bases were regenerated by adding ammonium chloride to the alkaline solution. The filtered, washed, and dried precipitate readily recrystallized in contact with methanol. It was washed with cold methanol in which it is moderately soluble (m.p. 135° C.), and recrystallized from a large volume of ether. Colorless fine needles melting at 135° C. were thus obtained. In admixture with F 24 it was completely liquid at 130° C. Found: C, 72.17; H, 7.03; N, 5.09; OMe, 20.64%. Calcd. for $C_{17}H_{19}O_3N$: C, 71.58; H, 6.67; N, 4.91; 2 OMe, 21.76%.

Cularine

There are certain alkaloids which are repeatedly encountered, and among these protopine is the only one which has been isolated from all plants in the Fumariaceae family which have been subjected to more than a cursory examination. Cryptopine and allocryptopine have each been found in six out of twenty species investigated by the author. Bicuculline has been found in nine species, and the new alkaloid, now referred to as *cularine*, has been isolated from five species; namely, *D. cucullaria*, *D. eximia*, *D. formosa*, *D. oregana*, and *C. claviculata*. It is expected that a communication dealing with the last named plant will be placed on record in the near future. Cularine crystallizes in large stout prisms only when it is quite pure, or if crystals for seeding are available, and until a method of purification was discovered it constituted the major portion of the uncrystallizable bases in the fraction (BC) from the plants mentioned. It was discovered, however, that it yielded a sparingly soluble acid oxalate and this could be recrystallized only from large volumes of hot water or methanol, and the alkaloid regenerated from the oxalate with excess alkali crystallizes readily from ether in which it is only moderately soluble. It melts sharply at 115° C. and shows $[\alpha]_D^{25} = +285^\circ$ ($c = 0.8$ in methanol). The simple formula $C_{20}H_{23}O_4N$, containing three methoxyl groups, is in good agreement with the analytical values, but some work on its degradation points to twice this formula. Calcd. for $C_{20}H_{23}O_4N$: C, 70.38; H, 6.75; N, 4.11; 3OMe, 27.27%. Found: C, 70.27; H, 6.90; N, 4.28; OMe, 27.32%.

Its presence in *D. eximia* and *D. formosa* is limited to several parts per million of the dry weight, and its isolation and definite identification was possible only with the relatively large amounts of these plants available. The properties of the oxalate greatly simplified the work. The other plants contain somewhat larger amounts.

The sparingly soluble oxalate obtained from *D. eximia* was a mixture from which the cularine salt was separated by extraction with hot water. The less soluble salt could not be redissolved in a reasonable amount of boiling water. It was dissolved in dilute hydrochloric acid, and the alkaloid regenerated by adding an excess of potassium hydroxide and extracting with ether. The washed and dried extract on evaporation to a small volume deposited a

new alkaloid in colorless fine needles. When recrystallized from ether, in which it is only slightly soluble, this alkaloid melted sharply at 102°C . It is alkaloid F 30 and is best represented by $\text{C}_{19}\text{H}_{21}\text{O}_4\text{N}$. Calcd. for $\text{C}_{19}\text{H}_{21}\text{O}_4\text{N}$: C, 69.74; H, 6.40; N, 4.28; 3OMe, 28.34%. Found: C, 69.75; H, 6.48; N, 4.59; OMe, 28.92%.

It is non-phenolic, and the presence of three methoxyls, as well as the analytical figures, indicates a close relation to cularine, of which it appears to be the N-desmethyl derivative. The amount of this alkaloid is limited to about 1 gm. in 35 kilos of dried plant. Nevertheless, Hoffmann degradation has yielded a small amount of base, characterized as the picrate, which appears to be identical with that of the methine of cularine.

Alkaloid F 21

The alkaloids in the mother liquor from the isolation of cularine and F 30 from *D. eximia* as oxalates were regenerated with alkali and extracted with ether. No crystals could be obtained directly from this product. It was treated with acetic anhydride, warmed gently, and allowed to remain in the ice chest for a week. Water was added and when the anhydride had disappeared the mixture was extracted with ether. The aqueous solution yielded a small amount of glaucine. The ether extract was freed of solvent and the residue heated for 36 hr. with methanolic potassium hydroxide. The basic portion was converted to hydrochloride in hot water. On cooling, a sparingly soluble hydrochloride, melting at 256°C . without effervescence but some darkening, separated in slender prisms. The free base was regenerated from this and crystallized first from ether and then from methanol-ether. Colorless rectangular plates melting at 80°C . were thus obtained. Alkaloid F 21 dissolves in cold sulphuric acid to form an orange colored solution, which on heating exhibits the following color changes:— yellow, green, blue, purple, and finally brown at a high temperature. The yield was 0.1 gm. from 35 kilos. Found: C, 66.74; H, 7.08; N, 3.97; OMe, 32.47%. Calcd. for $\text{C}_{20}\text{H}_{25}\text{O}_5\text{N}$: C, 66.85; H, 6.96; N, 3.90; 4OMe, 34.54%.

Cularidine

The fractions (BSE and EES) from *D. cucullaria* are relatively small. Each was neutralized with hydrochloric acid in methanol, and the concentrated solution treated with dry ether until the turbidity was permanent, and then with sufficient acetone to redissolve the precipitate. In the course of months a small amount of crystalline hydrochloride had separated from each fraction. This proved to be sparingly soluble in methanol or cold water, and was recrystallized from the former. The free base was liberated by means of ammonia and taken up in ether. The new alkaloid, *cularidine*, crystallized readily when the ether solution was evaporated to a small volume. It was recrystallized from methanol-ether and obtained in colorless fine needles melting sharply at 157°C . The analytical figures are in fair agreement with $\text{C}_{19}\text{H}_{21}\text{O}_4\text{N}$, containing two methoxyls, but the crucial observation that it yields cularine on methylation with diazomethane is more important.

It therefore is O-desmethylicularine. Calcd. for $C_{19}H_{21}O_4N$: C, 69.74; H, 6.40; N, 4.28; 2OMe, 18.88%. Found: C, 68.94; H, 6.57; N, 4.44; OMe, 18.59%.

Corydalis sempervirens

l-Adlumine (Alkaloid F 23)

This alkaloid, which previously has been referred to as alkaloid- η , and which was obtained from *C. scouleri* (12), has also been isolated from this plant. The fraction (BS), from which the greater portion of protopine and cryptopine had been removed, was heated with methanolic potash, water was added, and the methanol boiled off. The aqueous alkaline solution was decanted from the insoluble bases through a filter, and the filtrate treated with an excess of ammonium chloride. The precipitated base was filtered off, and redissolved in hot dilute hydrochloric acid. The base was reprecipitated with ammonia, filtered off, dried, and recrystallized from chloroform-methanol. Colorless six-sided plates of F 23 were thus obtained. It melted sharply at 180° C., and this melting point was not depressed when the alkaloid was admixed with a specimen from *C. scouleri*. The optical activity is opposite in sign but equal in degrees to that of adlumine from *A. fungosa*, namely $[\alpha]_D^{25} = -42.5^\circ$ ($c = 0.8$ in chloroform).

The *dl*-form of adlumine was prepared by dissolving 0.064 gm. of each of the *d*- and *l*-forms in chloroform and evaporating to a thin syrup. Methanol was then added and the remainder of the chloroform evaporated. On cooling, stout short prisms melting sharply at 190° C. were obtained. It is interesting to note that a mixture of approximately equal amounts of the *d*- and the *dl*-forms was completely liquid at 175° C.; this confirms the earlier observation that a mixture of the *d*- and *l*-forms had a melting point lower than that of either individual.

The hydrolytic oxidation with dilute nitric acid was carried out as with *d*-adlumine (5). Owing to the paucity of material only 0.2 gm. was available for the experiment. The acid fragment was converted by alkali treatment into the sparingly soluble 3:4-methylene-dioxy-phthalide which, when recrystallized twice from water, melted sharply at 232° C. either alone or in admixture with an authentic specimen. The basic fragment was also heated with alkali. The 1-keto-2-methyl-6:7-dimethoxy-tetrahydro-isoquinoline was isolated as previously described and recrystallized from ether. It melted at 125° C., and in admixture with an authentic specimen from adlumine (m.p. 126° C.) it melted at 125° to 126° C.

Alkaloid F 20

After the known alkaloids of *C. sempervirens* (3) were separated, all the mother liquors were combined, dissolved in dilute hydrochloric acid, filtered, and the bases precipitated by the addition of ammonia. The aqueous filtrate was extracted with ether and the extracts were evaporated to a small volume. The crystals which separated were recrystallized twice from methanol in which this base, F 20, is slightly soluble. Colorless prisms

melting at 221° C. were thus obtained. Calcd. for $C_{18}H_{23}O_6N$: C, 64.86; H, 6.91; N, 4.20%. Found: C, 64.42; H, 7.08; N, 4.37%. Methoxyl is absent.

It may be added that alkaloid- γ , previously reported from this source (6), has been identified as a slightly impure specimen of protopine.

Dicentra canadensis

Alkaloid F 22

In a previous communication dealing with this plant (1) the author recorded the isolation of a substance having the formula $C_{37}H_{38}O_{10}N_2$. It was regarded as neutral owing to its insolubility in hydrochloric acid. It has now been found that it yields a crystalline hydrochloride when its solution in chloroform-methanol is treated with a methanolic solution of hydrogen chloride. The pale yellow product obtained under these conditions is readily soluble in chloroform, only sparingly soluble in hot methanol, and virtually insoluble in hot water. It melts at 286° C. to an orange colored resin, sintering at a temperature several degrees lower.

The analyses are in good agreement with $C_{37}H_{39}O_9N_2Cl$. As in the free base, three methoxyl groups appear to be present. The loss of an oxygen atom is probably attributable to the formation of the chloride, rather than of the hydrochloride, from the base which is now regarded as quaternary. The base would then require the formula $C_{37}H_{40}O_{10}N_2$, which is also in satisfactory agreement with the analyses previously given. Calcd. for $C_{37}H_{39}O_9N_2Cl$: C, 64.30; H, 5.65; N, 4.05; Cl, 5.14; 3OMe, 13.47%. Found: C, 64.43; H, 5.77; N, 3.95; Cl, 5.69; OMe, 13.77%.

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A STUDY OF THE IRON IN A PODSOL SOIL BY MEANS OF AN IMPROVED DIPYRIDYL METHOD¹

BY W. J. DYER² AND W. D. MCFARLANE³

Abstract

An accurate determination of the ferrous and ferric iron content of a colored soil extract is possible by the direct application of the dipyridyl method when an Evelyn photoelectric colorimeter and a suitable light filter are employed.

It is shown that a sodium carbonate treatment of a podsol soil increases the water extractable iron and organic matter. The soluble iron is contained in the humic acid fraction. Since the iron in these extracts reacts quantitatively with $\alpha\alpha'$ -dipyridyl, it is believed to be entirely combined in a manner similar to that of the soluble unionized complexes of iron with hydroxy organic acids.

Part I. An Improved Method for the Determination of Ferrous and Ferric Iron in Soil Extracts

The reagent $\alpha\alpha'$ -dipyridyl reacts only with ferrous iron which is not bound to nitrogen, and has been used by Hill (3) and McFarlane (6) for the determination of iron. Recently Ignatieff (5) proposed a method for the determination of ferrous iron in soil solutions which employs this reagent. Aluminium chloride is used to decolorize the solution before the dipyridyl colorimetry is carried out, but the pink color of the ferrous dipyridyl complex is modified by this treatment.

The authors have developed a procedure, employing an Evelyn photoelectric colorimeter (2) and a 520 M light filter, by which accurate estimations of the ferrous dipyridyl color can be made even in humic acid solutions, without removing extraneous color. When this method is applied to a solution of the humic acid fraction or to soil extracts, the results indicate that in the presence of a reducing agent all the iron reacts with dipyridyl.

Experimental

An aliquot of the approximately neutral solution containing 10 to 20 γ of iron is placed in a 25 ml. volumetric flask. About 10 ml. of 10 *N* ammonium acetate solution (pH approximately 6.1) and 1 ml. of a 0.005 *M* solution of $\alpha\alpha'$ -dipyridyl containing 1.0 ml. of 1 *N* hydrochloric acid per 100 ml. is added. If ferrous iron is to be determined, the solution is diluted to 25 ml. with the acetate buffer, and mixed. To determine the total iron ($\text{Fe}^{++} + \text{Fe}^{+++}$) a trace of sodium hydrosulphite (iron-free) is added to reduce the iron, before the solution is diluted to 25 ml. After the solutions are allowed to stand, the color intensity is measured in an Evelyn photoelectric colorimeter, Filter 520 M being used. Usually the color attains its maximum intensity in a few minutes, but when large amounts of organic matter are present it

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may be necessary to let the solution stand for five hours before the color intensity is measured. To compensate for the color of the extract, the colorimeter is adjusted to read 100% transmission of light with a blank solution prepared in a manner identical with that of the preparation of the colored solution but to which no dipyrldyl has been added. The ferrous dipyrldyl concentration is then determined by reference to calibration data that have been previously prepared, as described below.

The approximate absorption curve of the ferrous dipyrldyl was determined with the Evelyn colorimeter from measurements made on the light transmission of the pink color at the different wave-lengths of light transmitted by different filters. The curve was similar to that obtained by McFarlane (6) by spectrometry, and shows the maximum absorption at about 520 millimicrons. Accordingly, the 520 M filter was used for the determination.

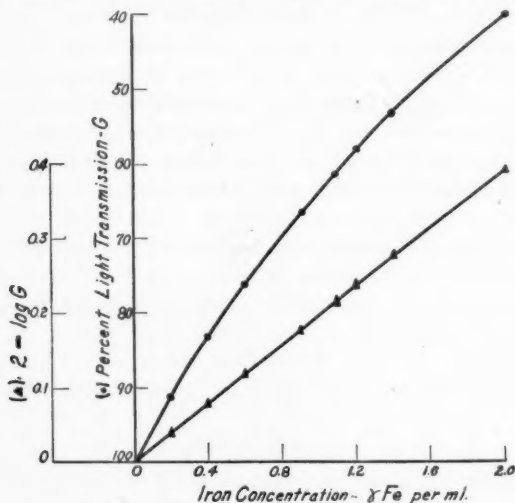


FIG. 1. The relation between the concentration of iron and the color intensity of ferrous dipyrldyl solutions.

Hill (3) states that the ferrous dipyrldyl complex is stable at pH values between 3.5 and 8.5. Since our method of measuring the color intensity is more sensitive than the methods formerly used, this point was investigated more fully. The results showed that the complex is undissociated in the pH range 3.2 to 8.2. Within this range of pH a very slight fading of the color with increasing acidity was noted, but the difference is not significant. The concentration of dipyrldyl in 1 ml. of the reagent is twice the theoretical amount required to react with 2×10^{-5} gm. of iron. This excess is in accordance with Hill's recommendations.

The calibration data were obtained by application of the above procedure to standard solutions of ferrous ammonium sulphate. A calibration curve may be plotted, as in Fig. 1, which shows the relation between photometer

readings and iron concentration, or, if Beer's law is applicable, a constant may be calculated from the relation

$$C = \frac{2 - \log G}{K_1},$$

in which C is the concentration, G is the corrected galvanometer reading, and K_1 is a constant. If K_1 is calculated from the readings with different concentrations of iron, it is evident that Beer's law will be applicable over the range in which the values for K_1 are constant. From the straight line relation between iron concentration and $2 - \log G$, as shown in Fig. 1, it is evident that K_1 is constant over the range plotted. By calculation, K_1 is found to be 0.197 ± 0.002 , with a maximum variation of 0.005.

Part II. The Organic Combination of the Iron Extracted by Alkali from Podsol Soil

When a typical Quebec Appalachian upland podsol soil is treated, in the autumn, with 1,000 lb. of sodium carbonate per acre, a marked increase in the yield of oats is obtained.* The oat straw has a bright golden yellow color and contains about 25 mg. of iron per 100 gm. of dry matter, whereas the straw from the untreated control plots has a rust color and an iron content of about 12 mg. per 100 gm. of dry matter.

Experiments are described below which show that a significant amount of iron can be extracted with alkalis from this podsol soil. When a sodium carbonate or pyridine extract of this soil is fractionated, practically all the dissolved iron is found in the humic acid fraction. Burke and co-workers (1) and Olsen (7) have shown that humic acid holds iron in solution at a pH greater than 6.0, and that iron so combined has a stimulating effect on the growth of plants and soil micro-organisms. Presumably the iron is combined with α -hydroxy acids to form soluble unionized complexes (Smythe and Schmidt (8) and Hopkins (4)). However, humic acid as obtained from soil always contains nitrogen, and it is of interest to know whether any of the iron in the humic acid fraction is linked to nitrogen. The authors' analysis by the dipyriddy method indicates that no significant amount of the iron in the humic acid fraction of this soil is bound in the form of an iron-nitrogen complex. Since all the iron reacts directly with dipyriddy, the dipyriddy method becomes particularly valuable for the rapid determination of ferric and ferrous iron in soil extracts. The results in general indicate that the beneficial effect of sodium carbonate on the oat crop is due, in part at least, to its rendering more iron available to the plants.

Experimental

To examine the effect of sodium carbonate on the soil, a number of glass percolators were set up each containing a kilogram of soil which had been treated with a known amount of a sodium carbonate solution. Percolation

* Unpublished data—Macdonald College Soil Fertility Committee, 1937.

was carried out immediately by pouring enough water on the surface of the soil to give one litre of percolate. Analysis of the percolates (Table I) showed

TABLE I
ANALYSIS OF WATER PERCOLATES OF SODIUM CARBONATE TREATED SOIL

Test No.	Amount of sodium carbonate, lb. per acre	pH	Organic matter, gm. per 100 gm. soil	Iron, mg. per 100 gm. soil
1	Nil	5.23	0.173	0.47
2	2,000	5.90	0.185	0.85
3	4,000	6.36	0.250	2.30

that the organic matter and iron content, and the pH of the percolates, increase with the amount of sodium carbonate added. Sodium carbonate, therefore, increases the solubility of organic matter and of iron.

To investigate further the effect of alkalis on the soil iron, extraction of the soil with sodium carbonate and with pyridine was next carried out. A sample of 100 gm. of the podsol soil was refluxed for four hours with 500 ml. of pyridine. The filtrate was evaporated nearly to dryness *in vacuo*. The residue was shaken with ether and water, and the voluminous precipitate which separated in the ether layer was filtered off and dried. The ether and water solutions were evaporated to dryness separately. Analysis of the three fractions (Table II) showed that 92.1% of the iron in the extract was contained in the humic acid precipitate.

TABLE II

Fraction	Organic matter, gm.	Total iron, mg. extracted from 100 gm. of soil
Ether residue	0.190	1.30
Water residue	0.371	0.33
Precipitate	1.283	19.00

In a second experiment, 100 gm. of soil, ground to pass a 100-mesh sieve, was extracted overnight by continuous shaking with 500 ml. of cold pyridine. The material was filtered; the residue was refluxed with 500 ml. of pyridine at 115° C. for four hours, and filtered. Each filtrate was

evaporated nearly to dryness *in vacuo*, the residue extracted with ether, filtered, and dried. The analysis of these humic acid fractions is shown in Table III, (1) and (2).

In further experiments, 20-gm. samples of the soil were extracted by continuous shaking with 500 ml. of a 1% sodium carbonate solution for 24 hr. One sample had been previously leached with 200 ml. of 1% hydrochloric acid solution. This treatment liberates the humic acid held as the insoluble calcium humate. The extracts were filtered and the humic acid was precipitated with an excess of 10% hydrochloric acid. The precipitates were dried and analyzed, with the results shown in Table III, (3) and (4).

TABLE III
ANALYSIS OF HUMIC ACID FRACTIONS

Extracting agent	Humic acid residues			Total iron in humic acid fraction of 100 gm. of soil, mg.	Iron in humic acid fraction as per cent of total soil iron*
	Weight, gm. per 100 gm. soil	Nitrogen, %	Iron, %		
Pyridine at 25° C. (1)	0.350	—	3.7	1.4	0.12
Pyridine at 115° C. (2)	0.804	1.98	19.4	19.4	1.64
Sodium carbonate—direct (3)	4.535	2.48	4.8	21.5	1.82
Sodium carbonate—after leaching with HCl (4)	6.550	2.62	14.4	94.0	7.97

* The total iron content of this soil is 1.18%.

Evidently, direct extraction with sodium carbonate or pyridine dissolves only a small amount, about 1.7%, of the total iron in the soil. Nevertheless, an increase of this magnitude in the available iron would be quite significant so far as plant nutrition is concerned. A comparison of the results in Tables I and III shows that direct percolation of the soil does dissolve a considerable amount (about 10%) of the total alkali soluble iron. Pretreatment with hydrochloric acid increases the proportion of the total iron which is subsequently extracted by sodium carbonate to about 8.0%. The total iron content of the humic acid fractions was determined by ashing a weighed quantity in a vitreosil dish at 500° C., dissolving the ash in 5 ml. of 6 *N* hydrochloric acid, and applying the dipyridyl method.

To determine whether all the iron in the humic acid fraction reacts directly with dipyridyl, the following experiment was carried out. A weighed portion, about 60 mg. of the solid, was dissolved in warm 5% ammonium hydroxide solution and filtered. The total iron in an aliquot of this solution was determined after ashing. Iron that reacted directly with dipyridyl was determined by adding the reagents plus sodium hydrosulphite to a neutralized aliquot of the solution as described above. The blank compensated for the color of the test solution, and complete recovery of added iron in such solutions could be obtained.

Examination of one humic acid preparation gave:—

	Micrograms of iron per ml. solution
Total iron (average of 12 estimations)	7.86 ± 0.30
Iron reacting directly with dipyridyl (average of 8 estimations)	7.87 ± 0.06

It may be concluded, therefore, that in this humic acid fraction, which contained 2.48% of nitrogen, no significant amount of the iron is combined with nitrogen.

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AN IMPROVED METHOD FOR THE DETERMINATION OF PHOSPHATE BY PHOTOELECTRIC COLORIMETRY¹

BY W. J. DYER² AND C. L. WRENSHALL³

Abstract

A sensitive and accurate method for the determination of phosphate, involving the application of the Evelyn photoelectric colorimeter to the ceruleomolybdate reaction, is described. This technique makes it possible to differentiate phosphate phosphorus from other forms of phosphorus.

Conditions affecting the rate and extent of color development have been studied. The results show that the maximum color intensity is developed in about five minutes after addition of the reducing agent. The determination may be made in the presence of extraneous color, and soil organic matter does not interfere with the reaction. Under the conditions specified, using light filters, Beer's law applies to the reaction in soil extracts as well as in pure solutions in the range 0.02 to 0.40 parts per million of phosphorus.

Introduction

The concentration of phosphate phosphorus in natural waters and soil extracts is often extremely low; consequently, methods of the highest sensitivity are required for its determination. Comparison of the stated sensitivities attained by the several procedures which have been proposed for the development of the so-called ceruleomolybdate blue color shows that the Truog and Meyer (11) method is capable of the highest sensitivity; it produces an appreciable blue color in the presence of 0.02 parts per million (p.p.m.) of phosphate phosphorus. This procedure is at present in widespread use for the visual colorimetry of phosphate in a variety of soil extracts. It has the advantage that the manipulations are simple and rapid. There is a justifiable general tendency to regard the results of this and other similar methods as rough estimations, valuable only for purposes of comparison. The characteristics of the reaction, which is subject to certain interferences, and which produces an unstable color that increases to a maximum intensity and then gradually fades, have apparently not been accurately defined. Furthermore, precise visual readings are not possible when the solution to be tested is already tinted, as soil extracts so frequently are.

The use of the single-cell type of photoelectric colorimeter* equipped with light filters, described by Evelyn (7), has enabled the writers to make a more exact study of the reaction than has been reported hitherto, and thus to define the conditions under which the concentration of phosphate phosphorus may be determined with a higher degree of accuracy. Determinations may be

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* This colorimeter, complete with filters, may be obtained from the Rubicon Company, 29 North 6th Street, Philadelphia, U.S.A.

carried out on highly colored soil extracts with virtually no impairment of accuracy. This permits the full realization of the aim of Parker and Fudge (10) who proposed the determination of inorganic phosphate phosphorus in the soil extract by direct colorimetry, total phosphorus after ignition, and, by difference, phosphorus present in forms other than orthophosphate, that is presumably combined in organic molecules.

Three recent papers (6, 8, 12) describe techniques for the determination of phosphate by photoelectric colorimetry. The higher sensitivity of the reaction employed in the present work, and the use of light filters selected with reference to the light absorption of the ceruleomolybdate blue color, lead to distinct advantages.

The reaction on which the method depends is a reduction of molybdate by stannous chloride in the presence of phosphate. In the reaction the blue compound, phospho-conjugated ceruleomolybdate, is formed. According to Deniges (4, 5) this compound has the formula $[(\text{MoO}_3)_4 \cdot \text{MoO}_2]_2 \cdot \text{H}_3\text{PO}_4 \cdot 4\text{H}_2\text{O}$. Reduction is effected in the presence of sulphuric acid of such concentration that the reduction of molybdate to the true molybdenum blue, $\text{MoO}_2 \cdot 4\text{MoO}_3$, is inhibited. Arsenate or antimonate, if present, act as chromogens in the same way as does phosphate.

Experimental

In the proposed method the blue color is developed in a suitably diluted aliquot of the solution to be analyzed, contained in a colorimeter tube, and the maximum intensity of the color is determined by the deflection shown on the galvanometer. The concentration of phosphate in the final solution may be obtained by reference to a calibration curve, or, since the reaction conforms to Beer's law, it may be calculated from the following relation:

$$C = \frac{2 - \log G}{K_1},$$

where C is the concentration, G the corrected galvanometer reading (tables of $2 - \log G$ are provided with the instrument), and K_1 is a constant. The calibration curve, or the constant K_1 , is previously established by using solutions of known phosphate concentration.

REAGENTS

The reagents used were those specified by Truog and Meyer (11), namely:

- (1) Acid molybdate reagent; 2.5% ammonium molybdate in 10 *N* sulphuric acid.
- (2) Stannous chloride; 25 gm. of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 100 ml. of concentrated hydrochloric acid, made up to one litre, and preserved from oxidation by means of a layer of mineral oil.
- (3) Standard phosphate; contains 5 p.p.m. of phosphorus as phosphate.

The acid molybdate reagent is stable for at least six months if protected from direct sunlight. The stannous chloride deteriorates slowly and should

be discarded after about one month. The reagents should give a satisfactory blank test. A phosphate solution of known strength should be tested daily to check reagents and other conditions affecting the calibration constant.

The proportions of sulphuric acid and molybdate have been thoroughly investigated by Truog and Meyer (11) and lately by Holman and Pollard (9); it has been found that the ratio recommended by the former was satisfactory.

PROCEDURE

An aliquot of sample solution is introduced into a 50 ml. volumetric flask, diluted with distilled water to approximately 45 ml., 2 ml. of acid molybdate reagent is added and the contents immediately mixed. Water is added to bring the volume to exactly 50 ml., and the contents is again thoroughly mixed. The dilution of the original sample should be such as to give a final concentration of phosphate phosphorus between 0.02 and 0.4 p.p.m. A 10 ml. aliquot is pipetted into a colorimeter tube placed in the colorimeter, and the rheostat so adjusted that the galvanometer shows a reading of 100, the 660 filter being used. One drop of stannous chloride reagent is then mixed with the contents of the tube, and the galvanometer reading is observed five minutes after the addition of the stannous chloride. The concentration of phosphate phosphorus in the final solution is obtained by reference to calibration data, and that of the original solution by taking into account the dilution of the sample.

The method of setting the blank corrects for any color in the solution that absorbs light passing the 660 filter; consequently, interference from extraneous colors in the sample solution is eliminated. The slight increase in volume due to the addition of stannous chloride need not be considered, as it is the same in standards and unknowns, and hence is included in the calibration.

If only small quantities of sample solution are available, the whole procedure may be carried out in the colorimeter tube, as follows: pipette 10 ml. of sample solution into a colorimeter tube, add 0.4 ml. of acid molybdate, and mix. Adjust to the blank setting, add stannous chloride, and determine the color intensity as directed above. For this technique, a special calibration should be made, care being taken that the standard phosphate solutions are tested in identical manner.

If a photoelectric colorimeter is not available, valuable work may be done with a balancing type visual colorimeter of large capacity, such as the Kennicott-Campbell-Hurley colorimeter*, or by nesslerization. The visual technique is applicable between the limits of phosphate concentration stated above, and the colors are not sufficiently intense to be matched in an ordinary colorimeter of small capacity. The colors should be developed simultaneously in the standard and in the unknown solutions, the directions of Truog and Meyer (11) being followed, and the comparison should be made five minutes after the addition of the stannous chloride. Visual comparisons are never entirely satisfactory, however, when the sample solutions are appreciably tinted.

* Obtainable from the Arthur H. Thomas Co., Philadelphia, U.S.A.

INVESTIGATION OF THE REACTION

Selection of the Color Filter

The approximate absorption curve of a blue solution prepared by the above-mentioned procedure was obtained by the use of a series of filters, each transmitting narrow portions of the visible spectrum. The percentage of the total light transmitted by the blue solution in each range of wavelengths was determined. The absorption curve is shown in Fig. 1. The peak of absorption occurs in the red near 660 millimicrons. Accordingly, the 660 filter, standard with the instrument, was used for this determination.

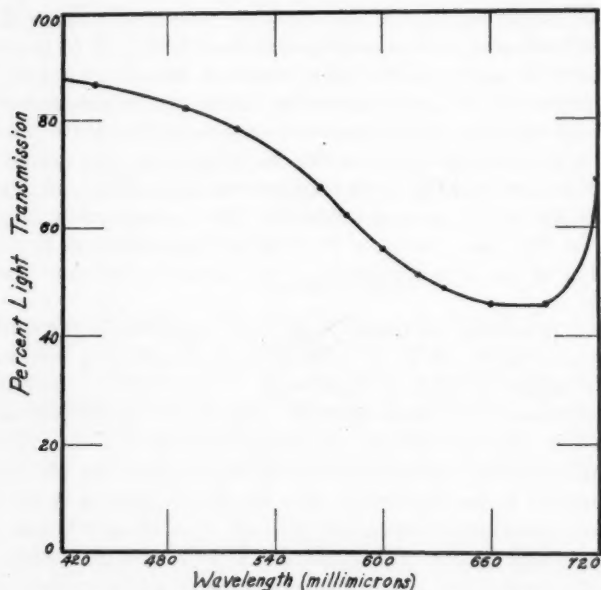


FIG. 1. Absorption curve of phospho-conjugated ceruleomolybdate blue.

Effect of Stannous Chloride

Chapman (2) has made a preliminary study of this effect, using glass color standards, and he noted that the intensity of the blue color was influenced by the amount of stannous chloride added. This point has been further investigated. A series of 10 ml. aliquots containing the required amount of acid molybdate reagent was prepared from each of three standard phosphate solutions, from a soil extract, and from pure water. Graded amounts of the stannous chloride reagent were added to each series, and the color development was measured. The results are shown in Fig. 2. The amount of stannous chloride used by Truog and Meyer (11) corresponds to the 0.03 ml. addition, and 0.05 ml. was the amount used in this investigation. It is evident that the amount of stannous chloride added has a definite effect

on the intensity of the color developed. Apparently the effect is due to reduction of the ammonium molybdate itself, since the color of the blank increases with increasing amounts of reducing agent just as does that of the solutions containing phosphate. The behavior of the soil extract is the same as that of pure solutions. Evidently the amount of stannous chloride added should be kept reasonably constant, although small variations from the recommended level will not introduce appreciable errors.

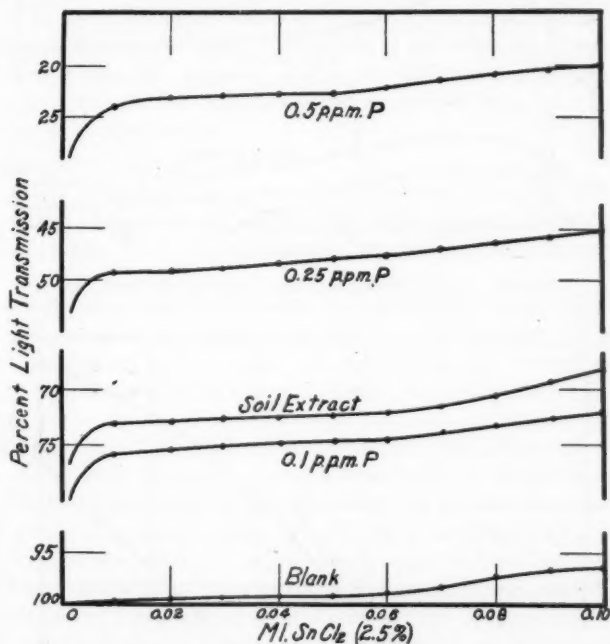


FIG. 2. Effect of stannous chloride on the color intensity.

Rate of Reaction

The rates of color development and fading for this reaction have not previously been accurately charted. Fig. 3 shows results obtained by measuring the intensity of the blue color in terms of percentage light transmission at several intervals after the addition of stannous chloride. A soil extract and standard solutions containing three different concentrations of phosphate were examined.

These results show that the maximum color is developed five minutes after the addition of stannous chloride. In solutions of lower phosphate content this maximum intensity is maintained for five minutes or more, but fading begins sooner at the higher concentrations. It seems likely that the earlier fading in the higher range is responsible for the fact that Beer's law is not

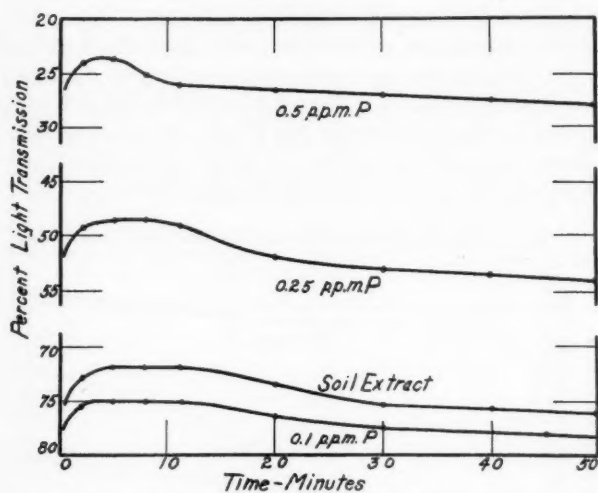


FIG. 3. Rate of development and fading of color intensity.

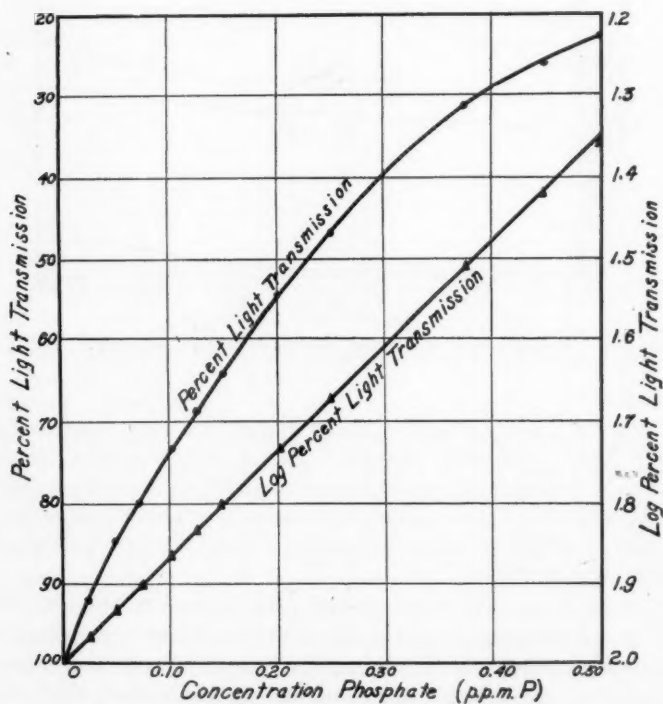


FIG. 4. Calibration curve and application of Beer's law.

applicable to solutions containing more than about 0.4 p.p.m. of phosphorus. It would also account for inaccuracies in techniques in which the time of reading is not rigidly standardized, and where comparisons are made in the range of concentrations greater than 0.5 p.p.m. of phosphorus.

The effect of permitting a considerable period to elapse between the addition of acid molybdate reagent and the addition of stannous chloride was investigated, pure phosphate solutions, phosphate solutions containing 5 p.p.m. of ferric iron, and water extracts of soils being used. A delay of as much as two hours in the addition of stannous chloride was without effect, but longer intervals caused an appreciable diminution of the intensity of the color. With soil extracts containing organic matter, it was found advisable to proceed directly, as a cloudiness sometimes developed on long standing after the addition of acid molybdate.

Calibration Curve and Beer's Law

Solutions of phosphate of known concentration were tested by the procedure described above; the resulting calibration curve is shown in Fig. 4, in which percentage light transmission is plotted against phosphate concentration.

The straight line relation between the logarithm of percentage light transmission and phosphorus concentration shows that in pure solutions ranging in concentration up to 0.4 p.p.m. of phosphorus, or somewhat higher, the reaction conforms with Beer's law. The constant, K_1 , has a value of 1.315 ± 0.014 .

Under reasonably uniform external conditions the calibration values remain constant over long periods. Wide fluctuations in temperature should be avoided; it has been observed that lowering the temperature of the solutions from 26° to 21° C. reduces the intensity of the blue color, with a corresponding decrease in the value of K_1 . Calibrations should be made for each instrument under prevailing operating conditions, and K_1 should be checked frequently.

The validity of the application of Beer's law to the determination of phosphate in soil extracts was tested. Known amounts of phosphate were added to soil extracts, determinations were made, and the amounts determined were compared with the known additions. Typical results are shown in Table I.

TABLE I
APPLICATION OF BEER'S LAW TO SOIL EXTRACT DETERMINATIONS

Added phosphate, p.p.m. P	Readings	Phosphate, p.p.m. P		Recovery, %
		Found	Increase	
Soil extract	93.75, 94.0, 94.0	0.020	—	—
0.025	87.0, 87.0, 87.0	0.045	0.025	100.0
0.050	81.0, 81.0, 80.75	0.070	0.050	100.0
0.100	69.75, 70.0, 70.0	0.119	0.099	99.0
0.100	69.75, 70.0, 69.5	0.120	0.100	100.0
0.150	60.5, 60.0	0.168	0.148	98.7
0.200	52.0, 51.75, 51.75	0.218	0.198	99.0
0.300	38.5, 37.75, 37.75	0.323	0.303	101.0
0.400	28.0, 28.0, 27.75	0.422	0.402	100.5

The recovery of added phosphate is seen to agree closely with the known additions. As a further test, soil extracts were diluted to different extents, and the concentration of phosphate in the original extracts was calculated from the concentrations in the final solutions. Results are given in Table II.

TABLE II
EFFECT OF ORGANIC MATTER (IN COLORED SOIL
EXTRACTS)

Extract	Aliquot (ml.) diluted to 50 ml.	Phosphate found, p.p.m. P	Conc. of phosphate in extract, p.p.m. P
(a)	10	0.098	0.490
	10	0.099	0.495
	20	0.202	0.505
(d)	20	0.023	0.057
	40	0.043	0.054
(e)	20	0.030	0.075
	40	0.062	0.077

From Tables I and II it is evident that the calculation based on Beer's law applies to soil extracts as well as to pure solutions. Table II shows that the extent of dilution of the original extract does not materially influence the result. This is of special interest in view of the statement of Holman and Pollard (9) that dissolved soil organic matter interfered with the reaction in such a way that higher results were obtained in the more dilute extract. This type of interference has not

been encountered here. We have noted one case in which the light transmission was interfered with by the formation of a colloidal precipitate which appeared on addition of the acid molybdate reagent.

INTERFERING SUBSTANCES AND CONDITIONS

The effect of the common inorganic acids and their salts has been adequately defined by Chapman (2) and by Holman and Pollard (9).

Organic Acids

Citric acid, which is commonly used in making soil extracts, inhibits the normal reaction, even when present in low concentration. Davies and Davies (3) found that citric, oxalic, tartaric, malic, lactic, and glycollic acids interfered with the Bell-Doisy and Fiske-Subbarow methods, and showed that citric and malic acids form molybdate complexes.

Table III records the effects of some organic acids on the color development in a phosphate solution containing 0.2 p.p.m. of phosphorus.

The data show that α -hydroxy acids, and oxalic acid, have a marked inhibiting action on the color development, while the other acids had little or no effect.

The successful application of the method to water extracts of soil indicates that interfering organic substances are seldom if ever present in concentrations high enough to affect the reaction.

Ferric Iron

Truog and Meyer (11) believed that ferric iron seriously interferes with the reaction by retarding the rate of color development and causing a greenish

TABLE III
EFFECT OF ORGANIC ACIDS

Acid	Acid conc. in final soln., %	Phosphorus, p.p.m., found at various time intervals after addition of stannous chloride		
		5 min.	10 min.	20 min.
Citric	0.9	0	—	—
	0.1	0.03	0.04	0.05
	0.02	0	—	—
Tartaric	0.2	0.052	—	0.065
Acetic, CH_3COOH	0.2	0.200	0.200	0.180
Glycollic, $\text{CH}_2\text{OH} \cdot \text{COOH}$	0.2	0.175	0.162	0.150
Oxalic, $\text{COOH} \cdot \text{COOH}$	0.2	0	0	0
Trichloracetic, CCl_3COOH	0.2	0.200	0.196	0.180
	2.5	0.168	0.172	0.162
Propionic, $\text{CH}_3\text{CH}_2\text{COOH}$	0.2	0.200	—	—
Lactic, $\text{CH}_3 \cdot \text{CHOH} \cdot \text{COOH}$	0.02	0.200	—	—
	0.4	0.100	0.125	0.125
Hydracrylic, $\text{CH}_2\text{OH} \cdot \text{CH}_2 \cdot \text{COOH}$	0.2	0.200	0.193	0.180
Malonic, $\text{COOH} \cdot \text{CH}_2 \cdot \text{COOH}$	0.2	0.200	0.200	0.186
Hydroxymalonic, $\text{COOH} \cdot \text{CHOH} \cdot \text{COOH}$	0.2	0	—	—

TABLE IV
EFFECT OF FERRIC IRON

Ferric iron added, p.p.m. Fe	Phosphorus found, p.p.m. P	Deviation	
		p.p.m.	%
0	0.195		
1	0.194	-0.001	-0.5
2	0.191	-0.004	-2.0
3	0.191	-0.004	-2.0
4	0.187	-0.008	-4.1
5	0.189	-0.006	-3.1
6	0.188	-0.007	-3.6
7	0.191	-0.004	-2.0
8	0.190	-0.005	-2.6
9	0.187	-0.008	-4.1
15	0.195*	0	0
20	0.198†	+0.003	+1.5
30	0.201†	+0.006	+3.1
40	0.196†	+0.001	+0.5

* Faded rapidly.

† Faded very rapidly with maximum color development at about four minutes.

tinge to develop, but stated that the effect was probably not serious if the concentration was less than 4 to 6 p.p.m. of iron. Under our conditions we have not found the effect to increase with increasing iron concentration to the same extent as did these workers, and a rapid rate of fading has been observed rather than a retardation of the color development. Table IV shows results obtained by applying the standard technique to solutions containing 0 to 40 p.p.m. of ferric iron, added as ferric chloride.

Evidently the color development was slightly inhibited in solutions containing 0 to 15 p.p.m. of iron; in solutions containing more than 15 p.p.m. of iron, the maximum color intensity was reached sooner, about four minutes after the addition of stannous chloride, and thereafter the color faded very rapidly. This makes high concentrations of ferric iron highly undesirable. Lower concentrations do not appear to introduce a serious error, but for accurate results the concentration of ferric iron should not exceed about 1 p.p.m.

In general, the writers have found that the iron content of soil extracts has not been high enough to cause interference at the final dilution.

Ferrous Iron

The general practice is to eliminate ferric iron by reducing it to ferrous iron, which has been considered not to interfere. Chapman (2) stated that ferrous iron may reduce the color development if the addition of stannous chloride is delayed after the addition of the acid molybdate. This point has been further investigated.

Ferrous iron was added to phosphate solutions in the form of a solution of ferrous ammonium sulphate. The stannous chloride was introduced at the desired time interval after the addition of acid molybdate. The results are shown in Table V.

TABLE V
EFFECT OF FERROUS IRON WITH DELAYED ADDITION OF STANNOUS CHLORIDE

Solution	Time interval between addition of molybdate and stannous chloride, min.	Phosphorus found, p.p.m. P	Deviation	
			p.p.m.	%
Blank + 1 p.p.m. Fe	1	0.0		
	2	0.0		
	5	0.0		
Phosphate soln.		0.191		
Phosphate soln. + 1 p.p.m. Fe	$\frac{1}{2}$	0.191	0.0	0
	$\frac{1}{4}$	0.186	0.005	2.6
	$\frac{3}{4}$	0.187	0.004	2.1
	1	0.185	0.006	3.4
	2	0.180	0.011	5.8
	5	0.162	0.029	15.2
Phosphate soln.		0.191		
Phosphate soln. + 2 p.p.m. Fe	$\frac{1}{2}$	0.185	0.006	3.4
	1	0.182	0.009	4.7
	2	0.173	0.018	9.4
	5	0.152	0.039	20.5
Phosphate soln.	1	0.183		
	2	0.183		
	5	0.183		
Phosphate soln. + 5 p.p.m. Fe	$\frac{1}{2}$	0.182	0.001	0.5
	1	0.163	0.020	11.0
	2	0.147	0.036	19.7
	5	0.132	0.051	27.9

It is evident that ferrous iron definitely inhibits the color development if any considerable time elapses between the addition of molybdate and the addition of stannous chloride. The effect is progressive with increasing time intervals. Even 1 p.p.m. of ferrous iron is sufficient to have an appreciable effect. With intervals of 15 sec. no effect could be detected. Evidently it is essential that the stannous chloride be added immediately (within 15 sec.) after the acid molybdate, if ferrous iron is present in the sample solution. In a previous paragraph it was noted that under normal circumstances intervals up to two hours do not affect the results.

PRECISION AND ACCURACY

The data of Tables I and VII, in which results of duplicate determinations are listed, show the high degree of precision attained by means of this technique. As there is no personal factor involved in making the readings, the same calibration may be used by different investigators without additional errors arising.

The accuracy of the method may be judged from data in Table I, in which the recovery of known amounts of added phosphate is reported.

The photometric technique has also been applied to the determination of total phosphorus in soil following fusion with magnesium nitrate, and in Table VI the results are compared with those of the standard A.O.A.C. titration of ammonium phosphomolybdate (1, p. 10).

TABLE VI
COMPARISON OF TOTAL SOIL PHOSPHORUS BY TITRATION AND COLORIMETRIC METHODS

Soil number	Total phosphorus, % of soil			
	Titration	Av.	Colorimetric	Av.
Macdonald College	0.1024 0.1019	0.1021	0.100 0.100	0.100
1	0.0798 0.0780	0.0789	0.072 0.073	0.0725
2	0.0946 0.0990	0.0968	0.094 0.099	0.0965
3	0.0782 0.0786	0.0784	0.078 0.077	0.0775

The results of the photometric method are in close agreement with those of the standard method, in spite of the high dilution factor involved. This indicates the high degree of accuracy that has been attained.

The method described has been used in this laboratory for the past six months in determining the phosphorus contents of a variety of soil extracts, and has proved highly satisfactory. A feature of this work has been the differentiation of inorganic and organic phosphorus on the basis that the

former reacts directly to give the blue color, while the latter reacts only after the organic matter has been destroyed by ignition. A series of results are given in Table VII.

TABLE VII
ORGANIC AND INORGANIC PHOSPHORUS IN SOIL EXTRACTS

Soil number	Phosphorus in extract, p.p.m. P						Organic phosphorus	
	Inorganic phosphorus			Total phosphorus				
	a*	b	Av.	a	b	Av.	p.p.m. P	%
Water extracts (1 : 5)								
1†	0.038 0.042	0.045 0.040	0.041	0.490	0.510	0.500	0.459	91.9
2	0.945 0.935	0.965 0.985	0.958	2.40	2.48	2.44	1.482	60.7
3	0.040 0.041	0.040 0.043	0.041	0.404	0.400	0.402	0.361	89.8
4	0.075 0.080	0.062 0.062	0.070	0.238	0.268	0.253	0.183	72.4
9	0.216 0.220	0.205 0.205	0.211	0.770	0.730	0.750	0.539	71.9
Acid extracts (1 : 200)								
1	0.034	0.035	0.034	0.085	0.080	0.082	0.048	58.5
2	0.106	0.110	0.108	0.140	0.146	0.143	0.035	24.5
3	0.072	0.073	0.072	0.097	0.106	0.101	0.029	28.6
4	0.141	0.141	0.140	0.135	0.145	0.140	0.000	0
9	0.280	0.254	0.267	0.277	0.262	0.269	0.002	0.8

* a and b represent duplicate extractions.

† Soil 1 is a Brown Forest; Soil 2, a Muck; Soil 3, a Calcareous Loam; and Soils 4 and 9 are Podzols.

In all the water extracts, and in most of the acid extracts, a considerable proportion of the phosphorus present appears to be combined in organic molecules. Thus there is an essential difference between procedures which determine the phosphorus of an extract after ignition and those which depend on direct colorimetry in the original solution, although this fact does not seem to have been generally recognized.

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